*Vol. 49, No. 4, November, 1960*

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BULLETIN OF

# EXPERIMENTAL BIOLOGY AND MEDICINE

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# INDUCED CHANGES IN SPONTANEOUS ACTIVITY IN THE RABBIT SPINAL CORD

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(Presented by Active Member AMN SSSR N. I. Grashchenkov)

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Original article submitted March 24, 1959

We have previously [3] investigated the electrical activity in the spinal cord of the rabbit at rest, as recorded by point-electrodes, and have described the method.

In order to determine the origin of the potentials and to interpret them, we have studied the effect on this activity of changes affecting both the inflow of impulses to the spinal cord and its functional condition. We here report the principal results observed in the lumbosacral region, though in many cases similar changes occurred in the thoracic region also.

## RESULTS

The effect of anesthesia. It is well known that the lower centers of the central nervous system are more resistant than the higher to the action of anesthetics. It would therefore be expected that during the early (light) stages, there would be a reduction in the descending flow of impulses into the cord. As the anesthesia becomes deeper, its effect on the spinal centers increases [12]. In this way the block of a spinal cord reflex increases in proportion to the depth of anesthesia.

We have investigated the changes in spinal potentials induced by ether. At the end of the stage of motor excitation, when it was not possible to record potentials on account of artifacts due to movements, the following changes were observed in the electrospinogram (ESG) and the electromyogram (EMG).*

Usually, the rapid oscillations were the first to suffer almost complete extinction. As a rule, the effect was better shown in the EMG. At this time, slow potentials were still recorded in the ESG, although their amplitude was sometimes somewhat reduced (Fig. 1). As the anesthesia deepened, the slow waves were also suppressed, and the recording consisted only of "noise" from the apparatus. Occasionally, at a certain stage, when the anesthesia was deepening or when the animal was recovering from it, slow potentials at the frequency of respiration were recorded from the lumbosacral region (Orbeli-Kunstman phenomenon) [1, 2, 3, 6]. An interesting phenomenon could be observed during the suppression of the rapid potentials under light anesthesia. At this time, regular peaks having an amplitude as high as 40-50 μv and a frequency of from 8 to 50 per sec were recorded. In the EMG, these peaks were either absent, or greatly reduced (Fig. 2). Like

* It was necessary to record both the ESG and EMG because the point-electrode measured potentials relative to an indifferent electrode inserted into an interspinal muscle, and it was important to consider the possibility of any muscle potentials being recorded [3].

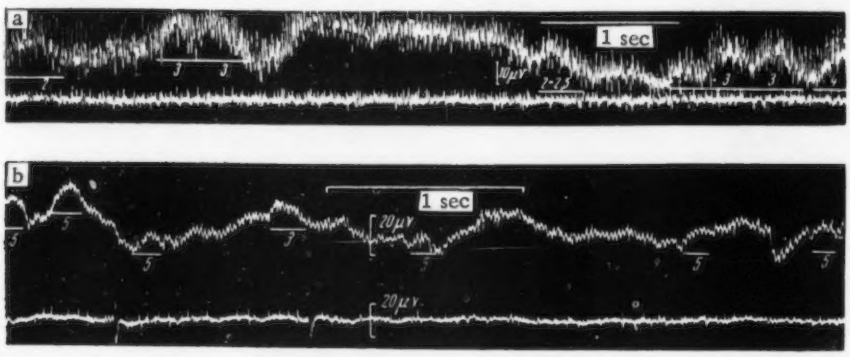


Fig. 1. The effect of anesthesia on the ESG of the lumbosacral region of the cord, and on the corresponding EMG. a) Initial trace; b) first stage of anesthesia. Curves, from above downwards: Time marker; ESG of lumbosacral region; EMG: figure indicate period of oscillation in cps.

Fig. 2. Regular spikes occurring in the ESG during anesthesia. Curves, from above downwards: Time marker; ESG of thoracic region; EMG.

all other electrical activity, these spikes disappeared as the anesthesia deepened. When the animal recovered from the anesthetic, the spikes gradually regained their initial high amplitude and were indistinguishable from EMG activity.

The effect of curarization. Curare-like drugs were used to reduce the flow of afferent impulses into the cord, the reduction affecting chiefly proprioceptor inflow; this in turn led to a reduction of motor impulses to the muscle, without any further operation being performed. We also found the effect of elatin, kondel'fin (preparation "K") and diplacin in doses whose central nervous action was not appreciable; the first two drugs, and especially elatin, were used in doses less than the amount required to eliminate voluntary movement. The injections were given intramuscularly as follows: Elatin – 10-15 mg (rabbit weighed about 2 kg), kondel'fin – from 1 to 3 $cm^3$ 5% solution, diplacin – from 1 to 3 $cm^3$ 2% solution.

The results are best described for diplacin, where the effect of the reduced afferent inflow were most clearly seen. This preparation had the least effect of the three, and there was a transitory reduction of muscle tone without complete loss of voluntary movement. No anoxia could occur through action on the respiratory musculature.

The electrical activity changed as follows. At first the high-frequency waves of both the ESG and EMG were suppressed. In the ESG, rather high amplitude regular spikes of up to 30-50 μv appeared, but these were not seen or else were greatly reduced in the EMG. The slow oscillations continued for a longer time. Sometimes, in the lumbosacral region slow oscillations at the respiratory frequency were reduced (Fig. 3). When the curarizing effect was stronger, the slow components of the ESG were also reduced.

Kondel'fin caused curarization of more sudden onset, so that some of the changes in spinal electrical activity observed when using elatin were sometimes missed. However, there was always a marked suppression of the rapid waves in both the ESG and EMG, and regular spikes appeared on the ESG together with smaller synchronized spikes on the EMG; the amplitude of the latter varied from zero up to an amplitude equal to the ESG spikes.

Diplacin had the strongest action, and when given in the dose reported above, rapidly caused respiratory disturbances, so that it was rarely possible to observe the early curarization stages.

The effect of interrupting conduction along the cord. Complete spinal thoracic section was carried out to eliminate the influence of higher nervous centers; it caused immediate cessation of electrical activity below the cut in the ESG and the EMG. In the acute preparation it was difficult to determine whether the cessation of electrical activity was due to interruption of descending impulses, or to spinal shock; observations could not be continued for long because the animals died rather quickly; (rabbits show little resistance to extensive damage to the central nervous system). Interesting observations were made on one rabbit having a spastic paralysis of the hindlimbs (the animal contracted the condition in the animal house,

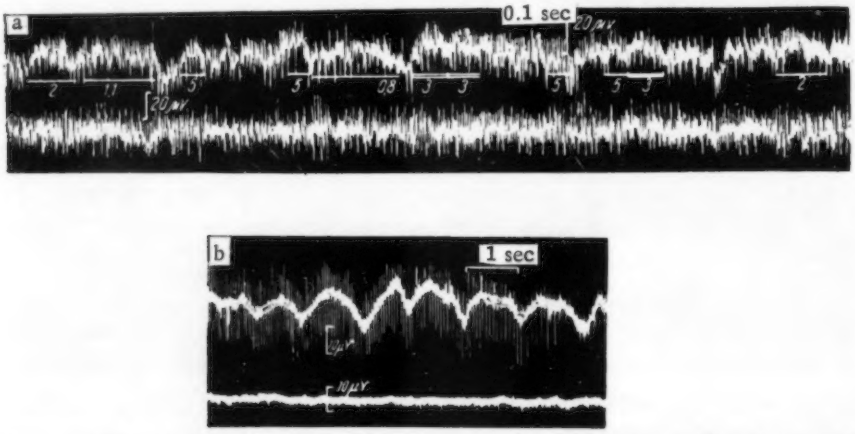


Fig. 3. Effect of curarization (elatin) on the ESG. a) Initial activity; b) early stage of curarization (appearance in the ESG of regular spikes, and slow oscillations at the respiratory rate). Traces and indications as in Fig. 1.

from unknown causes, not less than ten days before the experiment).†

The fact that there was a spastic paraplegia without any respiratory disturbance or paralysis of the front limbs showed that the damage to the cord must have been in the lower thoracic or upper lumbar segments. The absence of any movements, and the fact that there was no reaction ot painful stimulation of the hind limbs showed that conduction must have been completely or almost completely blocked.

The ESG and EMG records from the lumbosacral region when no stimuli were applied showed only very low amplitude slow waves. After stimulating the paralyzed hindlimbs by pinching or pricking, the characteristic slow waves returned to the ESG, and had a period of 500-200 msec, i.e., they resembled the δ-waves of the electroencephalogram. It is hardly possible to consider that the condition of spinal shock might have been present ten days after trauma had occurred, and the results obtained must therefore be ascribed to the reduced afferent inflow. Painful stimulation below the block appeared to make good the deficient inflow from the lower parts, and restored normal electrical activity in the lumbosacral region.

The effect of painful stimulation. The effect of additional afferent impulses to the lumbosacral region was particularly clearly shown when the recording electrode was inserted in the thoracic region of the cord. The increased inflow was caused both by painful stimulation caused by pricking the soft tissue and the meninges, and by mechanical stimulation of the substance of the cord by the electrode. Both these factors no doubt caused an increased flow of impulses into the lumbosacral region of the cord to pass along proprioceptor routes (or caused a response from the higher nervous centers).

The electrical activity of the latter changed quite consistently (registration was carried out after the animal had returned to the resting condition). There was an increase in the amplitude of both the slow and rapid waves (the latter also returned in the EMG) and there was a marked reduction in the period of the slow waves, and an increase in their rate. Whereas the initial activity showed low amplitude slow waves corresponding to type I activity, the stimulation described caused the amplitude to increase and the waves to be better defined.

When the slow type I waves were sufficiently large, painful stimulation might elicit slow components resembling type II waves [3].

The results obtained showed that in our experiments the potentials recorded from the cord were definitely related to the afferent inflow: Reduction in the number of either the afferent or the efferent impulses caused a reduction in spinal cord potentials; increased afferent inflow increased both the amplitude and the frequency of the potentials. We have therefore confirmed the results of Bremer [10], Ten Cate et al. [14, 16-18], Ward [19], and A. A. Oganisyan [4, 5].

On the other hand, the observations we made on the animal with paralyzed hind limbs, when there was no spinal shock, and the researches of Boels, Ten Cate, and Visser [9] do not confirm the findings of Mark and Gasteiger [15] who claimed an increase in the amplitude of potentials below the spinal cord section.

The regular high amplitude spikes in the cord which are found at a certain stage of anesthesia or curarization, and which are reflected in the EMG, are evidently due to synchronized activity of motoneurons. It appears that under these conditions synchronization represents a compensation for motor insufficiency; the insufficiency may result from the neuromuscular block caused by curarization, or from reduction in reflex excitation of the motoneurons in anesthesia. A similar explanation may be applied to the changes in the electromyogram which occur in cases of damage to the anterior horns [7, 13]. While it is difficult to explain the mechanisms of spinal and supraspinal synchronization, the fact that no such effect was observed after separation of the cord from the brain, and the results obtained by Alward and Fuortes [8] on the supraspinal mechanism of the synchronizing action of chloralose on the cord, indicate that the phenomenon we have observed is also mediated by a spinal mechanism; apart from our observations, there are only those of Bremer [10], which were obtained under light anesthesia and without decapitation.

In considering the spontaneous activity of the spinal cord, Bremer [10, 11] took the view that all activity in the central nervous system is of reflex origin, and he therefore failed to appreciate the qualitative differences between the spontaneous activities of the brain and spinal cord. However, this description is not adequate, because as far as general properties are concerned, it ignores the the undoubted differences between the mode of operation of the neuronal systems of different parts of the nervous system, due to their evolution into different functional organizations. If we consider the spontaneous activities of the cord and brain, it can be seen that they react to the inflow of impulses in different ways: in the brain, increased inflow causes a reduction in the amplitude of the slow potential waves, which recover when the inflow ceases, but in the cord, as has been shown above, these same potentials are proportional to afferent inflow.

Evidently, the difference between the spontaneous activity of the brain and that of the cord must represent a qualitative difference, although both are reflex in origin.

## SUMMARY

Animals were anesthetized or curarized, spinal cord block was established, and additional afferent stimulation applied; recordings were made of the spontaneous electrical activity of the lumbosacral region of the cord, and it was shown that the amplitude of the potential variations was directly proportional to the rate of arrival of impulses into the area from which the recording was made, and this

† Unfortunately no histological observations were made.

was specially true of the slow potentials. At the stage of light anesthesia or mild curarization spikes were generated at a steady rate; there were also slow fluctuations of potential at the respiratory rate. On the physiological conditions the spontaneous activities of the brain and spinal cord are differently affected by afferent inflow; it may therefore be suggested that though both are reflex in origin, the two activities are qualitatively different, and that different functional organizations of the two sections of the nervous system have evolved.

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‡ Original Russian pagination. See C. B. translation.

# THE EFFECT OF INSULIN HYPOGLYCEMIA ON FROG SPINAL REFLEXES

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(Presented by Active Member AMN SSSR V. I. Chernigovskii)

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At the present time a great deal of work is being done on the effect of hypoglycemia on the different parts of the nervous system. However as has been pointed out by E. Gel'gorn [1] very little information is available concerning the spinal cord. He quotes only one paper [5] in which it is reported that the tendon reflexes disappeared during insulin coma. Also, I. I. Gurevich [2] has shown that in the frog, spinal reflexes are reduced when hypoglycemia is induced by removing the viscera and maintaining the animal in a thermostat.

The object of the present investigation has been to determine the effect of insulin hypoglycemia on spinal cord reflexes in a warmblooded animal.

## METHODS

Cats were anesthetized with ether, and the spinal cord cut below the medulla. Heat was applied to maintain body temperature, and respiration sustained artifically. Recordings were made from a pair of antagonist muscles, the semitendinosus and the rectus femoris is response to stimulation of the central end of the peroneal nerve. A wide range of stimulus voltages was applied by a neon lamp stimulator at a frequency of 80 cps. Blood for analysis was removed from the femoral or carotid artery; sugar was estimated using Hagedorn-Hensen's method. Insulin and glucose were injected into the femoral or jugular vein.

When the preparation had been made, after an interval of half an hour, the threshold excitation of the peroneal nerve was determined at 15 min intervals. Every 60 min a record was made of the contraction of the muscle in response to a submaximal stimulus equal to 3-5 times the rheobase applied to the nerve, blood was withdrawn and the blood sugar measured. When there was a change in the reflex response, the measurements were made more frequently.

By continuously draining the wound with strips of gauze placed above and below the electrodes so as not to touch the nerve, and by fixing the electrodes firmly to a stand we were able to prevent fluid reaching the electrodes and to preserve the nerve undamaged. In control experiments on ten animals which were kept for 6-10 hours after making the preparation, both the threshold of reflex stimulation and the amplitude of contraction of the semitendinosus muscle in response to a submaximal stimulus showed no appreciable change (Fig. 1).

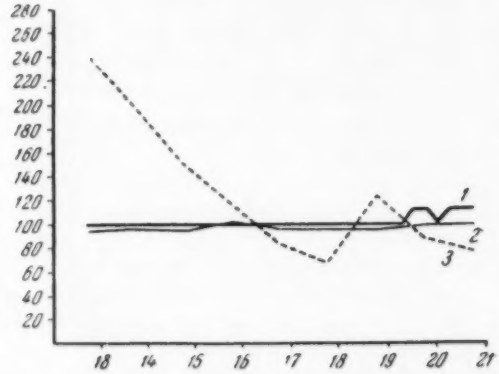


Fig. 1. Control experiment. The abscissa represents time of day in hours; ordinate: 1) Threshold stimulation of peroneal nerve, as percentage of original value; 2) amplitude of contraction of semitendinosus muscle in response to a submaximal stimulation of peroneal nerve, as percentage of original value; 3) blood sugar level (in mg%).

## RESULTS

In cats, 60-90 min after sectioning the spinal cord the blood sugar was 200-350 mg%, and it fell during the experiment reaching finally the value of 100 mg%. As can be seen from Fig. 1 the fall was smooth, it did not fall to a hypoglycemic level, and did not affect the threshold of excitation nor the contraction of submaximal stimulation. After the excitatory threshold had remained constant for 45-60 min, five or ten units per kg of insulin were injected intravenously.

In 35 of the 40 animals (87%), 3-6 hours after the insulin injection there was an increase in excitatory threshold and a reduced contraction of the muscle to submaximal stimulation (Fig. 2). The figure shows that as the blood sugar level falls there is a reduction in the contraction of the semitendinosus. The reflex was restored by injecting 4-10 ml of a 40% glucose solution. The recovery was maintained for 2-30 min in all the experiments except two, although it was not always complete.

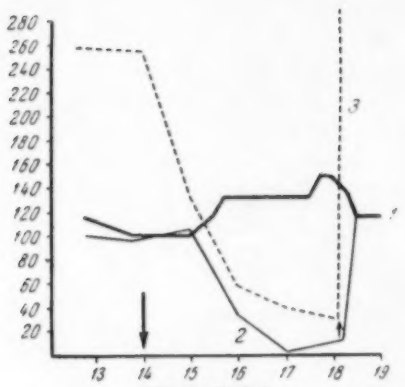


Fig. 2. Changes in the threshold of excitation and amplitude of contraction in response to a submaximal stimulus, after injecting insulin and glucose. Arrows indicate moment of injecting insulin (↓) and glucose (↑). Indications as in Fig. 1.

No case was observed of inhibition of the antagonist muscle caused by hypoglycemia.

It is important to note that in hypoglycemia the threshold stimulus changes less than the response to submaximal stimulation. Figure 2 shows that as the blood sugar level falls, the contraction to the submaximal stimulation falls almost 50 times, whereas the threshold value increases by only 50%, i.e., the condition resembles the equalizing phase of parabiosis.

The blood sugar level at which there was a considerable reduction in the reflex activity varied in different animals. In most experiments, the reduction occurred when the level fell below 50 mg%, and sometimes not until it had fallen to 25-30 mg%; however, in others the relfexes were reduced at a blood sugar level of 100 mg%. It is evident that the effect is not only caused by the actual blood sugar level but also by its rate of fall.

To find whether hypoglycemia affects chiefly the nervous system, the neuromyal junction, or muscular excitability, we arranged a set of experiments on 11 animals in which stimultaneous recordings were made of the reflex contraction of the semitendinosus muscle of one side, and the contraction in response to direct stimulation of the motor nerve, on the other.

Figure 3 shows the traces obtained in such an experiment, and it can be seen that as the blood sugar level falls, the reflex contraction is reduced, and that when glucose is injected, the contraction returns to its original value. There was no change in the response obtained to stimulation of the motor nerve. A similar effect was observed in seven other experiments. In the remaining three animals, the response to stimulation of the motor nerve was also reduced, but only at blood sugar levels below that which affected the reflex contraction, and the response returned more rapidly to normal after the glucose injection.

In most cases the threshold of excitation of the motor nerve increased during the experiment, but this increase cannot be attributed either to the reduction in blood sugar level or to its increase after the glucose injection, but was evidently a consequence of dividing the nerve. There was no reduction in the excitability of the muscle.

We may suppose therefore that the reflex changes are the result of altered conditions in the spinal cord itself.

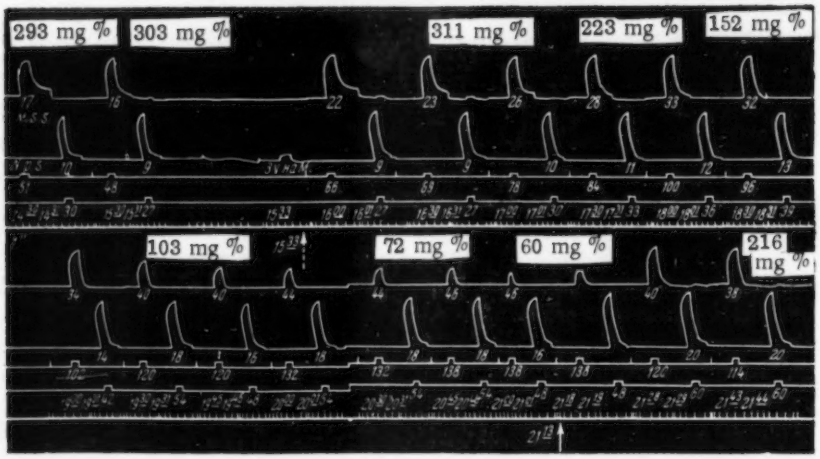


Fig. 3. Contraction of the semitendinosus muscle during hypoglycemia in response to stimulation of an afferent or a motor nerve. Curves, from above downwards: Blood sugar level in mg% (figures); contraction of left semitendinosus muscle; figures below curve represent threshold stimulus value; contraction of right semitendinosus muscle; stimulus marker of left peroneal nerve (afferent); stimulus marker of right semitendinosus nerve (efferent); time of day in hours and min; time marker (one second). The lower part of the curve is a continuation of the upper part.

Even during hypoglycemic convulsions, we found no increase in reflex excitability. Evidently the spasms are not caused in this way, but possibly result from an increased effect of proprioceptor influence on skeletal muscle [3,4].

Gell'gorn [1] has decided on the basis of published results that hypoglycemia may be a biphasic effect on the autonomic system, but only depresses the somatic nervous system, i.e., it acts monophasically.

In our experiments, we too have been able to observe only a depression effect. However, the phasic nature of the reaction is a very well established phenomenon, and it would certainly be premature to deny its existence in somatic nervous reactions. Under the conditions obtaining in our experiments, cat spinal reflexes were reduced by insulin hypoglycemia. The depression increased with greater degrees of hypoglycemia, but returned to normal after the glucose had been injected. We did not observe any reduction in the inhibition of the antagonist to be caused by hypoglycemia. Neuromyal transmission was either unaffected, or affected very much less than was the reflex response. There was no change in muscle excitability. Evidently the observed changes in the reflexes were due to alterations in the spinal centers.


## SUMMARY

Experiments on spinal cats in insulin hypoglycemia showed that spinal cord reflexes were depressed. They were restored by intravenous glucose injections. There was no phase of increased excitability nor was there any inhibition of the action of the antagonist muscle. The changes in the reflexes evidently originate in the central part of the arc, because myoneural transmission was not affected and muscle excitability remained unchanged.


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# THE MECHANISM OF THE EFFECT OF THE POSTERIOR ROOT GANGLIA ON SMOOTH MUSCLE

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A number of investigations [1, 4, 7, 9, 13] have been made of the effect of the dorsal root ganglia on smooth muscle. Nevertheless, much remains to be found concerning the mode of action of the posterior root fibers.

According to Kenkure [11], the dorsal spinal roots contain afferent parasympathetic fibers. L. A. Orbeli [8] has shown that in the dorsal roots there are autonomic fibers which make synaptic contact with the sensory cells of the ganglion. From the spinal ganglia nerve impulse spread antidromically along the the outgrowths from these cells.

A. V. Kibyakov [4] perfused frog hind limbs, and found that fluid taken from veins during excitation of the posterior roots was capable of dilating blood vessels of another frog. On this account it has been suggested that transmission of excitation in the posterior roots is mediated by acetylcholine.

An active acetylcholine-like substance has also been found in similar experiments by other workers [2, 10, 12].

Posterior root fibers which cause contraction of the canine intestine lose their motor influence after pancreas has been removed [3], and it has been found that there is then a disturbance to the formation of acetylcholine in the body [5, 6]. However, it has frequently been denied [14, 15] that acetylcholine is liberated during excitation of sensory nerves.

In the present work, an attempt has been made to determine the influence of the posterior roots on the smooth muscle of the urinary bladder, and on the m. retractor penis of the dog, and to find to what extent acetylcholine is involved.

## METHODS

The experiments were performed on dogs under ether-chloroform anesthesia. The animals were placed in position with the dorsal surface uppermost. The vertebral canal was opened at the level of the lower-lumbar and upper sacral segments. The spinal cord was exposed by a longitudinal cut in the meninges, after which a dissection was made at the dorsal roots which were then severed. The peripheral ends of the cut roots of the posterior two lumbar and anterior two sacral segments were ligatured and placed on buried electrodes. The wound was then closed by clips, and the animal was placed on its back, and the abdominal cavity opened. A purse-string suture was made at the base of the urinary bladder, an aperature was made in the wall and a rubber balloon was introduced into the cavity, connected to a U-shaped manometer by an air and water transmission system. A preparation was then made of the peripheral end of the m. retractor penis, which lay in the perineal region extending to the midline as a cord consisting of two thick bundles. The peripheral end of the muscle was cut and joined through a system of two pulleys to a lever recording on a smoked drum. Stimulation was applied from an induction coil supplied by a 2 v from a storage cell.

## RESULTS

In the first set of experiments, stimulation of the fourth and fifth lumbar dorsal roots caused the bladder to contract. The contraction caused the height of the curve to increase to three times or more the normal height (Fig. 1a). In some experiments the effect was weak, or did not occur at all. This result was evidently due to the initial motor activity of the bladder, on which the effect of dorsal root stimulation was superimposed. In these experiments, the so-called spontaneous activity of the bladder usually did not occur.

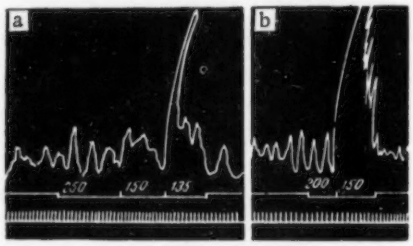


Fig. 1. Change in the contractions of the urinary bladder. a) On stimulating dorsal roots; b) same, after injecting eserine.

The response of the m. retractor to stimulation of the fifth lumbar and first two sacral dorsal roots was the opposite of that shown by the urinary bladder, and in each case there was a marked relaxation of the muscle (Fig. 2a).

To determine the mechanism of the action of the dorsal roots on the bladder and m. retractor, a second set of experiments was carried out in which the dog received

2-3 ml of 1 : 10,000 eserine and 6 mg per kg body weight of cocaine.

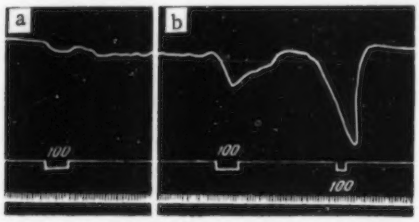


Fig. 2. Change in the contraction of the m. retractor. a) On stimulating the 1st lumbar dorsal root; b) same, after injecting eserine.

Stimulation was applied to the dorsal roots 30 min after injecting eserine. In all the experiments, when the eserine was injected for $1\frac{1}{2}$-2 hours there was both a greater contraction of the bladder (Fig. 1b) and a greater relaxation of the m. retractor (Fig. 2b). In cases where there had been no effect was always well shown after. In this way, the excitability of the dorsal roots was considerably enhanced. After injecting cocaine, dorsal root stimulation had no effect either on the retractor muscle or on the bladder.

The results of the experiments have shown that dorsal root stimulation causes responses from the urinary bladder and m. retractor to occur. The reaction is enhanced by eserine injection, but not influenced by cocaine. The results indicate that acetylcholine is involved in the mechanism of transmission of impulses via the dorsal roots. In this way acetylcholine comes to play an important part in the dorsal root activity as affecting different structures such as the urinary bladder and retractor penis muscle.

## SUMMARY

The posterior roots of the lumbar and sacral regions of the dog spinal cord were stimulated, and the effect on the smooth muscle of the urinary bladder and m. retractor penis found. The wall of the bladder contracted and the m. retractor penis relaxed. The reaction was intensified by eserine but was not affected by cocaine. Therefore, acetylcholine plays a significant part in the action of the posterior roots on these muscles.

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# THE CARDIAC COMPONENT OF CONDITIONED DEFENSIVE REFLEXES IN DOGS

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Cardiovascular conditioned reflexes in man and animals have been investigated in two different ways.

Some investigators [4] have studied the formation of conditioned cardiac and vascular reflexes in response to different extro- and interoceptor stimuli, while others [2, 3, 5] have studied the cardiovascular system and its participation in various complex conditioned reactions involving defensive movements, feeding, etc. In this way it has been shown that cardiovascular changes accompany any complex conditioned reflex act.

All positive conditioned feeding and motor responses are associated with an increased heart rate, and when the responses are inhibited, the heart rate is slowed. Nevertheless, at the present time a large number of problems concerning the structure of conditioned reflexes and their interrelationships remain unsolved. For instance it is not known to what extent the autonomic system is involved in the different features of conditioned reflex activity such as strength relationships, differential inhibition, disinhibition, and positive and negative induction.

We have made a study of heart-rate changes in positive and inhibitory motor responses to electrical stimulation.

## METHODS

The experiments were carried out on four male dogs weighing 14-20 kg in whom a set pattern (stereotype) of defensive reflex movements had been established. The conditioned stimuli used were a bell of moderate strength, a 96 w lamp, a buzzer, a metronome at 120 beats per min for the positive, and another at 60 beats per min for the inhibitory signal. The signals were applied for ten seconds; threshold electrical stimulation (50 c/s 0.8-1 mA) was applied during the seventh second. During the application of the conditioned and unconditioned stimuli, and also during the period between them, records were made of the motor response, respiration, and electrocardiogram (using an ink writing electrocardiograph). The R-R intervals were measured from the ECG, and plotted graphically to show the variation in heart rate.

## RESULTS

The animals represented different types of higher nervous activity. It was possible to observe the relationship between the motor responses and changes in heart rate for the different types. Dogs which judged by general excitability and the strength of the excitatory and inhibitory processes were of the two extreme types, showed reactions as follows:

In Blanche, who belonged to the weak inhibited type, the heart rate was slower than in the others and had a well-marked sinus (respiratory arrhythmia). While the animal was in the experimental chamber, before the beginning of the experiment the R - R interval lay between 0.54 and 0.84 sec. In Tarzan who was of the excitable ("unrestrained") type, the heart rate was rapid, and there was no sinus arrhythmia. The R - R interval had an average value of 0.32 sec. In Storm, excitation predominated, while in Rusty it was relatively weak, and these two dogs were therefore of an intermediate type. In Storm, before the experiment, the R - R interval varied from day to day from 0.42-0.44 to 0.46-0.50 sec, while in Rusty the variation was from 0.42-0.46 to 0.72-0.98 sec.

In all the dogs, after applying positively conditioned signals the R - R interval decreased, and in Storm and Rusty the sinus arrhythmia disappeared. In Blanche, the positively conditioned signals had only a mild accelerating action on the heart. For instance, in Storm and Rusty the R - R interval was reduced from 0.42-0.44 to 0.30 and from 0.42-0.46 to 0.32 sec respectively in response to the sound of a bell, while in Blanche, the same stimulus reduced the interval from 0.54-0.84 to 0.50-0.82 sec.

In each dog, the accelerating action of the positively conditioned signals was shown to a different extent, depending on the strength of the stimulus. The greatest effect, which was accompanied by enhanced respiration, was produced by the bell and the buzzer, while the least response was produced by light. The results showed that the ranking sequence of the stimuli was the same for the cardiac as for the motor and respiratory responses. For example, in Storm (experiment of June 20, 1958). the bell caused the R - R interval to fall from 0.46-0.50 to 0.30 sec, while the light stimulus reduced it from 0.50-0.54 to 0.38 sec.

It should be noted that in Tarzan in whom the heart rate was high before the experiment, the effect on the heart rate was less. Reinforcing the positive conditioned reflex as a rule further increased the rate.

The results of the effect on heart rate of inhibitory (differentiated) stimuli were particularly interesting. In Storm, Blanche, and Rusty, the inhibitory signal slowed the heart, and the effect was greater when differentiation of the motor response was complete. For instance, in Rusty (experiment June 20, 1958), the inhibitory metronom signal changed the R - R interval from 0.32-0.34 to 0.52 sec.

In Tarzan, in whom the differentiation had been elaborated with great difficulty and was very unstable, the inhibitory metronome signal of 60 beats per second, which had become completely differentiated with respect to the effect on the motor response (though it caused a marked inspiration), the heart rate was increased. It was interesting that in the remaining three dogs, as soon as the inhibitory signal was removed, the heart rate increased, whereas in Tarzan it was slowed.

The effect of conditioned defensive reactions was also studied in dogs in whom conditions reflex activity was disturbed.

It is well known that one of the commonest manifestations of conditioned reflex disorder is a disturbance of the quantitative relationships between stimulus and response. It was found that this relationship was also disturbed in so far as the heart reat was concerned.

In Rusty (experiment on July 1, 1958) after injecting 0.03 mg/kg of proserin, there was a phasic response, and a paradoxical motor reaction. The heart rate was similarly affected. Instead of the expectedly greater increase in response to the bell than to the light, the greater increment in rate was caused by the light, when the R - R interval fell from 0.54-0.60 to 0.36 sec, while when the bell was applied the interval was reduced only from 0.52-0.54 to 0.40 sec. The normal stimulus-response relationships were maintained for respiration (Fig. 1AB).

As has been shown above, in all the dogs positively conditioned signals caused an increase in heart rate corresponding to the motor and respiratory responses to the conditioned signal. However, in Storm the conditioned response to the bell was reversed (experiment of July 8, 1958). As a rule, while the animal was in the chamber before the beginning of the experiment and before the first bell-signal had been presented, it was very restless and breathing and heart beats were rapid. When the animal was in this condition, sounding the bell caused an increased inspiration and a gradual reduction of the motor response and, more important still, the slowing of the pulse (the R - R interval increased from 0.28 to 0.48 sec); there was thus a paradaxical relationship between the cardiac response and the signal given (Fig. 1C). When the bell signal was given three minutes later, it caused a tachycardia, the R - R interval being reduced from 0.28 to 0.24 sec.

The experiment performed on Storm on June 19, 1958 will serve as an example of a case where there was a different cardiac response to the conditioned signal and

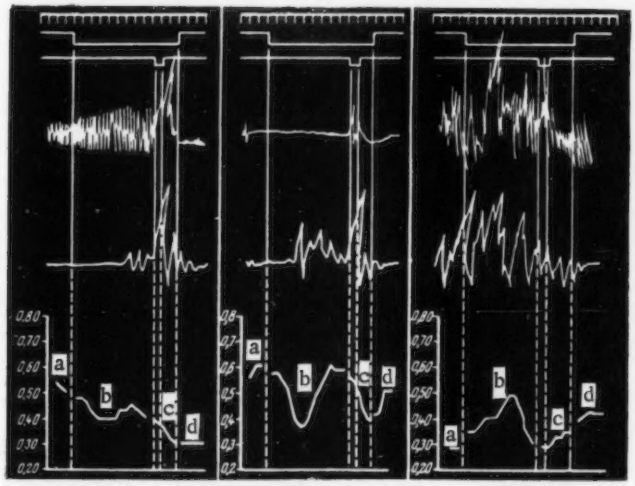


Fig. 1. Changes in heart rate when the normal stimulus-response relationship was disturbed. A) In response to bell; B) in response to light (Rusty - experiment of July 1, 1958); C) in response to bell (Rusty - experiment of July 8, 1958). a) Before presenting the conditioned signal; b) during seven seconds application of the conditioned signal alone; c) after reinforcement with electrical stimulus; d) after the cessation of the conditioned stimulus. Curves, from above downwards: Time marker (4 sec); conditioned reflex marker; unconditioned stimulus (electrical) marker; respiration trace; motor response; graph showing changes in heart rate (R -R interval).

where there was also failure (inhibition) of the motor response, and a weakened respiratory reaction to a light stimulus. These changes were associated with a marked shortening of the R - R interval from 0.5 to 0.38 sec.

Because any conditioned reflex (in this instance the defensive reflex) is made up of somatic and autonomic components it gives a considerable amount of information on the functional condition of the subcortical autonomic centers; we therefore carried out a set of experiments designed to determine the relationship between change in heart rate and the condition of adrenergic mechanisms of the adrenergic autonomic ganglia.

For this purpose an injection of 1 mg/ kg aminasin was given 15-20 min before the experiment. The effect was to suppress the motor conditioned response and to inhibit respiration.

During the period of application of the positive conditioned signals, the heart rate changed characteristically. In all the dogs, aminasin reduced it. Although the conditioned positive signals then produced little or no motor response, they did reduce the bradycardia caused by the aminasin injection; when the conditioned signals ceased, the heart rate was once more reduced (Fig. 2A, B). When the motor and respiratory responses were not completely eliminated, the reduction of the bradycardia was better shown (Fig. 2B).

Applying the inhibitory metronome signal of 60 beats per sec after giving aminasin further increased the bradycardia caused by the drug. When the inhibitory stimulus ceased, the heart rate again increased (Fig. 2C). The extent of the change in heart rate differed in the different dogs.

In Blanche who had a slower pulse and a stronger sinus arrhythmia than the other dogs, the inhibitory metronome signal of 60 beats per min increased the R - R interval from 0.50 - 1.44 to 0.50-1.72 sec. But in Tarzan, in whom aminasin caused but little reduction in heart rate, the reduction caused by the inhibitory metronome signal fell much less, the R - R interval increasing only from 0.46-1.0 to 0.46-1.14 sec.

It can be seen therefore that the effect of conditioned stimuli of different strengths and significance will produce consistent changes in the heart rate, and that their degree and nature will depend to a large extent on the type of nervous system possessed by the animal and on its general level of excitability.

It has been shown that the normal stimulus-response relationships and the nature and the stability of the differentiation of the motor response have their counterpart in heart rate changes. On the other hand, in cases of disturbance to the higher nervous centers, there was a failure of correspondence between heart rate changes and the strength and significance of the conditioned stimuli, and the responses of the different components of the conditioned reflexes were not consistent.

It was also shown that when the motor and respiratory components were inhibited by blocking the adrenergic mechanisms of the reticular formation by giving aminasin [1], the condition signals were still effective in producing heart rate changes, and also retained their signalling significance, so that after aminasin bradycardia, positive conditioned signals increased the heart rate, and negative reduced it.

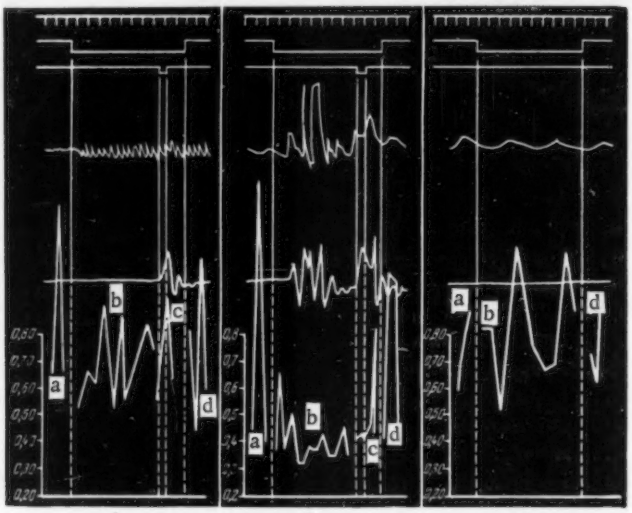


Fig. 2. Changes in the heart rate caused by positive and inhibitory signals applied after injecting aminasin. A) In response to bell; B) in response to buzzer (Storm, experiment of June 21, 1958); C) in response to metronome at 60 beats per sec (Rusty, experiment of June 21, 1958). Indications as in Fig. 1.

The results show that a study of the autonomic and somatic components of conditioned reflexes and their interaction, besides being of physiological interest are also of importance in connection with many pharmacological investigations.

An animal in which a defensive conditioned reflex having well-shown autonomic and somatic components has been elaborated is an extremely convenient model for the study of various substances affecting the central nervous connections, and the peripheral effects of central (cortical) excitation.

## SUMMARY

A study was made of the cardiac component of conditioned positive and inhibitory reflexes. Experiments were performed on four dogs in which a set of conditioned reflexes had been developed.

Changes in heart rate occurring during the presentation the conditioned signals were largely determined by the type of nervous system and by the general degree of excitation. The signalling significance of the conditioned stimuli, the strength-stimulus relationship, and the nature and stability of the differentiation of the cardiac response were consistent with the motor conditioned reflex.

Finally, it was shown that when the motor are respiratory responses were inhibited by 1 mg/kg aminasin, the conditioned signals were not only effective in causing heart-rate changes but also retained their signalling significance: when applied during aminasin bradycardia, positive signals increased and inhibitory decreased the heart rate.

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# AN AGGRESSIVE REACTION EVOKED IN DOGS BY A PROLONGED UNILATERAL APPLICATION OF ACID

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It has been shown in K. S. Abuladze's Laboratory that when a lengthy stimulation is applied to one side of the tongue, a stable residual (latent) excitatory zone is formed [2, 3, 4]. As a rule, the excitation is concentrated, and does not spread to centers on the opposite side.

We have investigated the effect of repeated applications of an acid stimulus to one side, the radiation of the excitation, and the development of a strongly aggressive response.

## METHODS

The experiments were carried out on two dogs, Pepper and Tsiskari, both of which of the excitable type. Tsiskari showed a well-marked defensive reflex. Unilateral stimulation was applied to one side of the exposed portion of the tongue [1]. The conditioned stimulus was a metronome, which was presented for 20 seconds and reinforced by stimulation of the left half of the tongue by decinormal hydrochloric acid for 30 seconds. Records were made of the salivary secretion on both parotid glands, which was recorded in scale divisions; the conditioned response was measured during a 20-second application of the conditioned stimulus alone, and the unconditioned response was measured 1 min after the onset of application of the unconditioned stimulus.

## RESULTS

Table 1 shows the results of two experiments performed on January 6, 1955, and May 21, 1956, and it can be seen that in response to a metronome reinforced by stimulation of the left side of the tongue, a conditioned reflex occurs only on the left side. With repeated application of the stimuli, on the day of the experiment the unconditioned secretion on the left side was somewhat in-

TABLE 1. Results of Experiments on Dogs, Performed January 6, 1955, and May 21, 1956

| Time | Interval (in min) | Conditioned stimulus | No of combined applications | Time lag (in sec) | Unconditioned stimulus | Parotid gland: left: conditioned secretion | Parotid gland: left: unconditioned secretion | Parotid gland: right: conditioned secretion | Parotid gland: right: unconditioned secretion |
|---|---|---|---|---|---|---|---|---|---|
| Experiment of January 6, 1955. Dog Tsiskari. | | | | | | | | | |
| 11 sec 10 min. | — | Metronome | 20 | 20 | HCl, N/10, on left side of tongue | 6 | 130 | 0 | 30 |
| 11 » 14 » | 4 | » | 21 | 20 | » » | 8 | 137 | 0 | 20 |
| 11 » 18 » | 4 | » | 22 | 20 | » » | 10 | 142 | 0 | 17 |
| 11 » 22 » | 4 | » | 23 | 20 | » » | 11 | 145 | 0 | 15 |
| Experiment of May 21, 1956. Dog Pepper. | | | | | | | | | |
| 11 » 10 » | — | » | 45 | 20 | HCl, N/10 on left side of tongue | 17 | 165 | 0 | 58 |
| 11 » 4 » | 4 | » | 46 | 20 | » » | 22 | 160 | 0 | 35 |
| 11 » 8 » | 4 | » | 47 | 20 | » » | 20 | 160 | 0 | 37 |
| 11 » 22 » | 4 | » | 48 | 20 | » » | 20 | 170 | 0 | 35 |

creased, while on the right it was decreased; there was no "intermediate" secretion.

The experiment was repeated on both dogs for four months, after which, in Pepper, in response to the conditioned stimulus of the metronome, a secretion appeared on the right side, i.e., on the side opposite to that which received the unconditioned stimulus. When the left side of the tongue was repeatedly stimulated, there was no reduction in the secretion on the right side, but it was actually increased.

There was an "intermediate" secretion from both sides. In Tsiskari, the same results were found, but during the experiment the animal moved about very restlessly, whimpered, snarled, and tried to bite the experimenter.

Table 2 shows typical results of two experiments made on June 10, 1955, and October 5, 1956.

Table 2 shows that now in both dogs the secretion frequently occurred not only on the left but also on the right side. When the unconditioned stimulus was repeatedly applied to the left side, secretion from the right side was not reduced, as in the previous experiments, but actually increased. Secretion occurred on both sides in between stimulations. In addition, Tsiskari behaved aggressively. These results show that when unilateral stimulation with acid is prolonged, the excitation is irradiated.

When similar experiments were repeated on Tsiskari, the restlessness and aggressive behavior were enhanced. The dog bit the experimenter and the assistant who led him into the room. Frequently, when tied on a lead in a room, the dog became so excited as to break her lead and attack people she had never seen before. The aggressive reaction became so strong that it became necessary to discontinue the application of acid conditioned and unconditioned stimuli, and to feed the dog with a meat mixture, on the stand and then to develop feeding conditioned reflexes. When food stimuli were given, the dog became much quieter and when the experiment was repeated with conditioned and unconditioned food stimuli, the previous aggressive behavior disappeared completely.

Usually, when conditioned stimuli were applied and were reinforced on one side of the tongue only, the conditioned reflex developed only on that side. When unilateral unconditioned stimuli were applied, salivary secretion occurred chiefly on the same side, though there was also a small amount from the opposite side. Repetition of the unconditioned stimuli led to a decreased secretion on the opposite side. In this case, when the metronome

TABLE 2. Results Obtained on June 10, 1955, and October 5, 1956

| Time | Conditioned stimulus | No. of coincidences | Time lag (in sec) | Unconditioned stimulus | Parotid gland: left: conditioned secretion | Parotid gland: left: unconditioned secretion | Parotid gland: right: conditioned secretion | Parotid gland: right: unconditioned secretion | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| Experiment of May 10, 1955. Dog Tsiskari. | | | | | | | | | |
| 11 sec. 30 min. | Metronome | 234 | 20 | HCl, N/10, on left side of tongue | 8 | 170 | 0 | 50 | Dog whimpered. |
| 11 » 34 » | » | 235 | 20 | » | 9 | 185 | 0 | 45 | Dog growls and rushes about. When the collecting bulb is removed, dog attempts to bite. |
| | | | | | Intermediate secretion | | | | |
| 11 » 38 » | » | 236 | 20 | » » | 10 | 195 | 5 | 70 | |
| | | | | | Intermediate secretion | | | | |
| 11 » 42 » | » | 237 | 20 | » » | 15 | 185 | 6 | 80 | |
| Experiment of October 5, 1956. Dog Pepper. | | | | | | | | | |
| 11 » 25 » | » | 254 | 20 | HCl, N/10, on left side of tongue | 20 | 155 | 7 | 40 | — |
| 11 » 29 » | » | 258 | 20 | | 28 | 165 | 10 | 55 | — |
| | | | | | Intermediate secretion | | | | |
| 11 » 33 » | » | 259 | 20 | » » | 25 | 170 | 10 | 70 | — |
| | | | | | Intermediate secretion | | | | |
| 11 » 37 » | » | 260 | 20 | » » | 27 | 180 | 8 | 85 | — |

was applied and was reinforced by stimulation of the left half of the tongue, secretion occurred not only on the left but also on the right side; when the unconditioned stimulus was applied repeatedly to the left side, there was no reduction in the secretion from the right side, which actually increased. Also, there was some salivary secretion from both sides occurred between stimuli.

Therefore, in this experiment , when unilateral acid stimuli were applied, in the balance between the basic nervous processes in the acid center were disturbed and the excitation irradiated. Probably the excitation evoked by the acid stimulus on the tongue was so powerful that inhibition was ineffectual against it. It appeared that in the excited animals, prolonged unilateral acid stimuli cause a breakdown in the inhibitory process which restrains the localized excitation at the acid center; this breakdown in turn leads to irradiation of the excitation, not only in the acid center of the ipsilateral side but also in that of the opposite side. When repeated unilateral stimuli are applied to dogs of the excitable type having well-developed defensive reflexes, irradiation of the excitation includes not only the secretory but also the motor links of the complex unconditioned defensive center. The result is a very aggressive response. In these cases, when the acid stimuli are discontinued and the dog is fed on the stand and conditioned feeding reflexes are developed, the agressive behavior ceases. In this way, the development of a zone of excitation in the feeding center facilitates inhibition of the increased excitability in the defensive center. A strong region of excitation in an antagonist center inhibits the aggressive reaction center by negative induction.

## SUMMARY

Prolonged unilateral application of acid to the tongue by K. S. Abuladze's method to two dogs of the excitable type produced an irradiation of the excitation, due to breakdown of the inhibitory process, so that the focus of the acid center remained strongly excited. The excitation spread to the acid center of the opposite side. In dogs with a strongly developed defensive reflex, the excitation irradiated from the acid center to the motor defensive center, and so provoked an aggressive reaction. The latter could be inhibited by the creation of a strong focus of excitation in the food center.

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*Original Russian pagination. See C. B. Translation.

# EFFERENT PATHS OF THE ESOPHAGO-INTESTINAL REFLEX

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We have previously described [1] reflex effects on the motility of the small intestine which can be elicited by stimulation of the receptors of the esophagus.

It was found that the reflex could be produced after sectioning the intestine rostral to the portion from which the recording was made, and this procedure eliminated the possibility of the excitation being transmitted along the walls of the gut; we concluded that the effect must be transmitted through lengthy reflex paths.

A number of studies of the innervation of the esophagus have been made [5-10, 12], and these describe afferent pathways which would account for the reflexes; in the present investigation we have studied the afferent pathways of the esophago-intestinal reflex.

## METHOD

Chronically esophagotomized dogs were used. The esophageal receptors were stimulated by the inflation of a thin-walled rubber ballon attached to the nipple of a pipe introduced through the artificial aperture. Intestinal movements were recorded on a kymograph operated by pressure changes in the balloon. In two dogs the reflexes were recorded before and after transdiaphragmal section of the vagi while the splanchnic nerves were left intact; in one dog, recordings were made before and after section of the splanchnic nerves and removal of the lumbar sympathetic chains, while the vagi were intact; in a third experiment, in two dogs, both vagi and splanchnic nerves were cut. In all, 122 experiments were performed. In addition, 48 tests were applied in 16 experiments by injecting 0.15-0.3 mg per kg of atropine in order to eliminate parasympathetic transmission.

## RESULTS

In the animals with the nerve supply to the gut intact, the balloon was inflated with air to a volume of 40-50 ml; when the esophagus was relaxed, the pressure required was from 2 to 10 mm, and when contracted, from 8 to 30 mm; when the intestine was relaxed or in a state of mild contraction, the effect was to produce contractions or to increase the strength of any that were already taking place (Fig. 1). When the intestine was already contracting vigorously, inflation of the esophagial balloon had no noticeable effect. In some experiments, the balloon was inflated to 75 ml, which required a pressure of from 5 to 20 mm when it was relaxed, and from 30 to 45 mm when contracted; in others, 100 ml were introduced at a pressure of 10-35 mm when the esophagus was relaxed or of 50-60 mm when contracted; if the intestine had previously been relaxed, tonic contractions now occurred. Inflation up to 75-100 ml caused still more powerful contractions to occur. Inflation to 100-150 ml during

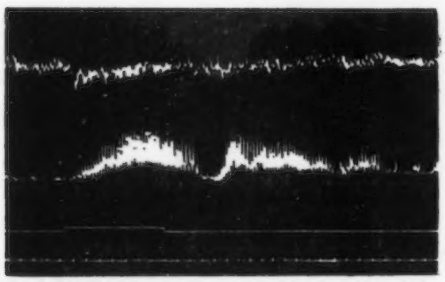

Fig. 1. Reflex excitation of movements of the small intestine caused by inflating a balloon to 50 ml in the esophagus of a dog with intact gastric and intestinal nerve supply. Curve, from above downwards: Gastric contractions; contractions of the jejunum; stimulus marker; time marker (15 sec).

a period of intestinal motility, caused the average amplitude of the movements to be reduced on account of changes in intestinal tone, which, however, could not be attributed to any inhibitory effect.

In dogs treated with atropine, and in whom intestinal movements had ceased, inflating the balloon to 50-75 ml produced no effect (Fig. 2). Because large concentrations of atropine block Auerbach's plexus and other cholinergic systems, it is not entirely justifiable to deduce that the excitatory effect is transmitted only by parasympathetic nerves. We therefore studied the efferent paths after cutting off the nerve supply to the gastrointestinal tract.

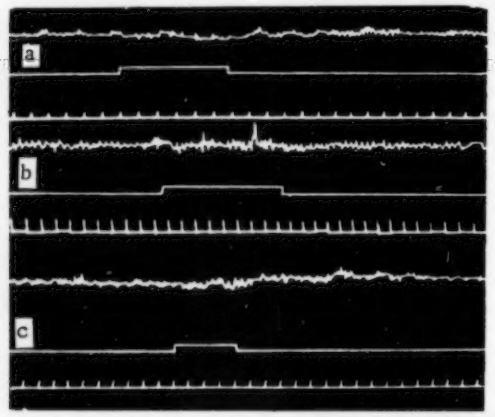


Fig. 2. Absence of reflex excitation of intestinal movements by esophagus. a) In atropinized animal; b) in the dog Bimba after sectioning the vagi; c) ditto in Laddie. Curves, from above downwards: Contractions of jejunum; stimulus marker (75 ml air); time marker (15 sec).

Stimulation of the esophageal mechanoceptors after dividing the splanchnic nerves and removing the lumbar sections of the sympathetic chains produced approximately the same motor response from the small intestine as it did in dogs with an intact nerve supply.

After the vagi had been divided, stimulating the esophagus by inflation to 40-50 or 75-100 ml when the intestine was at rest, caused no contractions in the latter (see Fig. 2). In some experiments, inflating the balloon to 75-100 or 150 ml during the latter third of a rest period caused some reduction in gastric and intestinal tone to occur at the time of inflation. The effect was better shown when the stimulus was applied during a period of weak intestinal movements. In Bimba, in three out of 18 experiments, after the vagi had been divided and immediately after the stimulus had been removed and the gastric and intestinal tone had increased, intestinal movements occurred. A dissection confirmed that both the vagi had been divided completely above the diaphragm. The contractions occurring after vagal section can not therefore be attributed to incomplete section of these nerves. It occured as a result of a rapid increase in gastric and intestinal tone, which in the latter third of a rest period are regularly excited. The rapid increase in gastric and intestinal tone occurring after the end of the excitation causes intestinal contractions to occur.

In Bimba, the reduction in gastric and intestinal tone observed during the esophageal stimulation could be explained as being due to an inhibitory effect mediated by the splanchnic nerves after the vagi had been divided. It is less likely that the inhibition is produced by a myenteric reflex.

After bilateral transdiaphragmal vagal section no reflex effects occurred between esophagus and small intestine, even when strong stimuli were applied. In some of the experiments, stimulating the esophageal receptors by inflating the balloon to 75 ml after the vagi had been divided but the splanchnic nerves left intact caused some inhibition of intestinal contractions, but when the balloon was inflated to 100-150 ml, in most cases, a marked inhibitory effect was observed (Fig. 3). These results indicate that the inhibitory effect from the esophagus is transmitted through the sympathetic (splanchnic) nerves to the small intestine. This hypothesis does not exclude the possibility that inhibitory effects are transmitted from the esophagus to the upper part of the small intestine by sympathetic fibers running in the vagi.

Confirmation that both excitatory and inhibitory influences are transmitted through the nerves to the gastrointestinal tract is afforded by experiments on dogs in which both vagi and sympathetic trunks were divided. Of the 14 experiments on the two dogs in which the lumbar sympathetic chains had been removed and vagi and splanchnic nerves divided, no single case was observed of any influence being transmitted from the esophagus to affect movements of the small intestine, even though the balloon was inflated up to 100-150 ml. These results afford further confirmation that in the dog Bimba the transmission of inhibitory effects resulting from esophageal stimulation during a period when the stomach and intestine were at rest does not result from inhibitory effects being transmitted by the myenteric reflex.

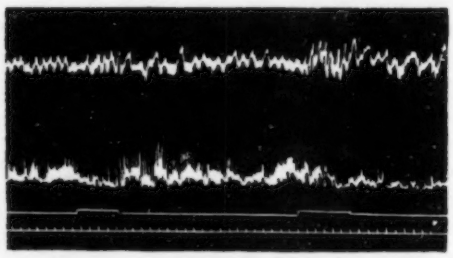

Fig. 3. Effect of esophageal stimulation on intestinal movements in the dog Laddie, after section of the vagi. Curves, from above downwards: Gastric contractions; contraction of jejunum; stimulus marker; time marker (15 sec).

The results indicate rather that when an adequate stimulus is applied to the esophageal receptors, reflex excitation of movements of the small intestine takes place and is transmitted through the vagi. When esophageal stimulation is increased, in addition to reflex excitation of small intestinal movements mediated by the vagi, reflex inhibition may also be transmitted by the sympathetic supply. This effect is not usually observed, because the excitatory effect predominates. The inhibitory influence is however well shown after vagal section.

It is known that strong stimulation of the esophageal receptors causes vomiting [1]. The reaction involves a number of other reflex mechanisms, and the process cannot be followed beyond the stage of inhibition of movement of the small intestine. The inhibitory effect may be shown when there is a sudden large inflation of the esophageal balloon. It also occurs if the dog chokes on a lump of food.

Douglas and Mann [11] have pointed out that there is no convincing evidence that the vagi play any part in mediating intestinal reflexes. The only demonstration of their motor function has been made on acute preparations. The results of our work reported in the present and in previous communications [2-4], which have been made on chronic preparations have shown that reflex excitation of movements of the small intestine is transmitted by the vagi.

## SUMMARY

It was shown that in chronically esophagotomized dogs, when the splanchnic nerves are left intact, the excitatory effect on movements of the small intestine produced by esophageal stimulation are eliminated by bilateral transdiaphragmal vagotomy, but are still present if the vagi are left intact and the splanchnic nerves divided. After division of both the vagi and the splanchnic nerves, stimulation of the esophagus does not influence movements of the small intestine. In animals with divided vagi and intact splanchnic nerves, pronounced inhibition of intestinal movements is caused by esophageal stimulation whereas when both vagus and splanchnic nerves are intact, the inhibition seldom occurs and it is thought that the reason for the failure is the prevalence of the excitatory effect of esophageal stimulation. It is concluded that the excitatory effect from the esophagus (esophagointestinal reflex) is transmitted through the vagi, and the inhibitory influence through the sympathetic nerves.

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# THE MECHANISM OF AMYLASE SECRETION IN THE DOG'S INTESTINAL JUICE FOLLOWING THE EXCLUSION OF PANCREATIC SECRETION

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In a number of publications G. K. Shlygin and co-workers of the Laboratory of Digestive Physiology [2-3] have shown that the characteristic intestinal enzymes - enterokinase, alkaline phosphates, saccharase, and others, are secreted into the intestine in the form of "mucous" globules. The "mucous" globules (the so-called solid portion of intestinal juice) are formed during the secretory process by the sloughed-off intestinal epithelial cells. After the breaking up of the globules, the enzymes are found in the liquid portion of the secretion. Due to this secretory mechanism the concentration of a number of characteristic intestinal enzymes in the solid portion of the juice is much greater than in the liquid portion.

In contrast to the above, amylase that is found in the dog's intestinal juice in very small quantities shows particular properties. It is found in liquid and solid portions of intestinal secretion in either the same quantities, or in the solid portion one and one-half or sometimes twice as much as in the liquid portion.

A supposition was advanced [4] concerning the possibility of transfer of varying quantities of dog amylase from blood into the intestinal juice. Investigation of dog salivary amylase [1] has established that when pancreatic ducts are ligated pancreatic amylase can be transferred into the saliva.

We have undertaken the problem of clarifying whether the presence of amylase in the intestinal secretion is the result of its active synthesis by intestinal glands, or whether it is due to transfer from the blood.

In order to solve this question we investigated the amylase content of blood and intestinal secretions of dogs with intact pancreas and in dogs without exocrine pancreatic secretions. In the dog, pancreatic juice is a very potent source of amylase; thus, after its exclusion one would anticipate a shift toward compensatory intestinal production of amylase, that is, if the intestines can produce it. If the intestines cannot produce it, then one can anticipate that the increase in blood amylase will render its transfer into the intestinal secretions, and in the latter case the amylase content of the intestinal juice should be closely related to blood amylase content.

## METHOD

The experiments were conducted on 12 dogs, each one of which had two loops of small and large intestine isolated, according to the method of Thiry. Eight of these animals were given a common ration, sufficiently rich in carbohydrates, four were given a predominantly protein ration poor in carbohydrates. The caloric content of both rations was 90 calories per kilogram of animal weight. Supplementation with vitamins and mineral salts was conducted in relation to the dog's requirements [5]. Intestinal juice was collected for 18 hours of periodic secretion after five hours of feeding. We recorded the weight of solid and liquid fractions, the amylase content, and likewise the content of enterokinase, alkaline phosphatase, lipase, peptidase, and saccharase. The amylase content of the juice and blood serum was determined by the modified method of Volgemut and the photometric method of Smith and Roe [6]. For the determination of other intestinal enzymes the methods accepted and partially worked out by the Digestive Physiology Laboratory of the Nutrition Institute of the Academy of Medical Sciences of the USSR [3] were used.

After the determination of the starting enzyme levels in the intestinal juice and in the blood of these animals, the exocrine pancreatic secretions were removed by means of ligating and cutting the efferent glandular ducts and by the isolation of the gland from the intesting by the omentum. The insulin secretory apparatus was not disturbed; this was ascertained by morphological investigations and through the determination of blood sugar.

## RESULTS

Comparison of the amylase content of both portions of intestinal secretions with blood serum has shown that the blood of a dog with intact pancreas is almost twice as rich in amylase as is the intestinal juice.

After ligation of the efferent pancreatic ducts (Fig. 1), one day after operation there is already observed an extraordinarily sharp increase in the blood amylase content (20 to 30 fold). At the same time there is a rapid increase (10 to 20 fold) in the amylase content of intestinal juice

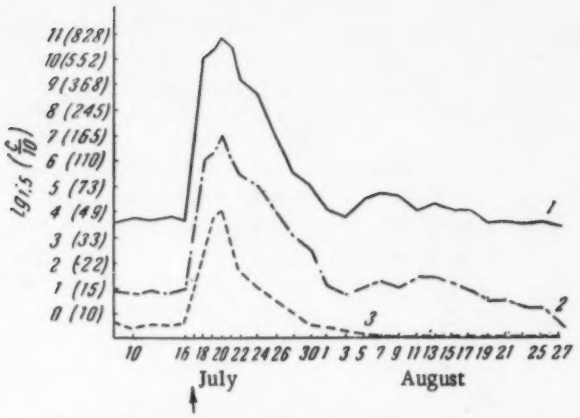


Fig. 1. Changes in amylase concentration after ligation of pancreatic ducts. 1) In blood; 2) in duodenal secretion; 3) in secretion of the large intestine. The arrow points to the time of duct ligation.

from the isolated loops of the duodenum and the large intestines. Gradually during one and one-half to two and a half weeks the content of amylase in blood and intestinal secretions becomes decreased, holds steady for 30-45 days, and then is decreased gradually. After four to five months, as a result of atrophy of the exocrine elements of the pancreas, the amylase content of blood and juice is 30-35% lower than before ligation. However, even at the end of the observations (eight to ten months after ligation) the concentration of blood amylase amounts to 40-45% of the starting level. The duodenal amylase content at the same interval after ligation is found to be a very low level. In the secretion of the isolated loop of the large intestine after one to one and one-half months after duct ligation, amylase can not be determined.

In Fig. 2, together with the changes in concentration of intestinal juice amylase, the contents of other enzymes are presented: enterokinase, alkaline phosphatase, peptidase, and saccharase. The heights of the starting levels of the given enzymes in the juice prior to ligation are taken as 100%.

Ten days after ligation of the ducts, when amylase concentration in the juice is still at an elevated level, the content of other enzymes is still unchanged. Two months after ligation of the ducts the amylase content of intestinal juice approaches the starting level; the content of other intestinal enzymes begins to increase.

At longer intervals, six to eight months after ligation, the amylase content in the juice is only one third, or less, of the starting level, whereas the content of enterokinase

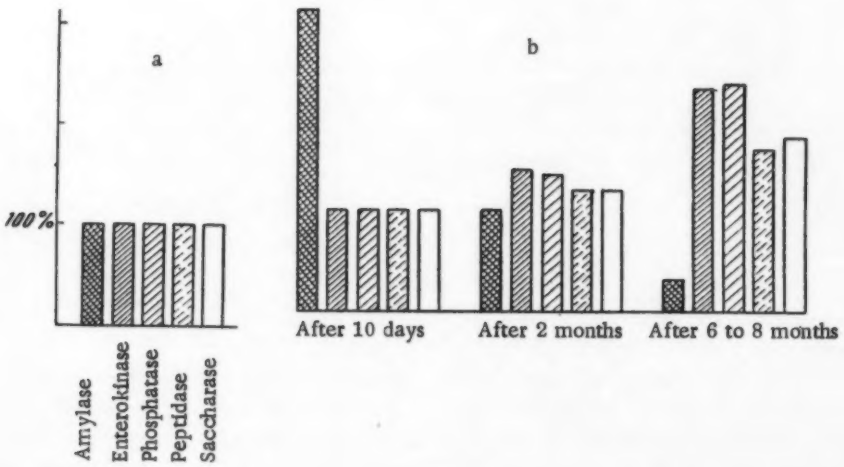


Fig. 2. Enzyme content of duodenal secretion (isolated loop). a) Prior to ligation; b) after ligation of pancreatic ducts.

and phosphatase is markedly increased and is two to two and one-half fold higher than the starting level. The peptidase and saccharase contents are also increased, approximately one and one-half fold higher than the original level prior to ligation. The increase in the production of a number of characteristic intestinal enzymes (enterokinase phosphatase, peptidase, saccharase) can be looked upon as a compensatory change in activity of intestinal glands.

The steadfast decrease of amylase in the digestive juice and in blood (as a result of pancreatic atrophy) leads to a drastic deficit of amylase in the digestive tract.

Naturally, a common ration that is generally rich in carbohydrate is unfavorable. Dogs receiving this ration lose more weight more rapidly than animals maintained on a carbohydrate poor and predominantly protein ration. Sharp deficit of amylase in the digestive tract after ligation of the glandular ducts leads to gastrointestinal disturbances in carbohydrate digestion, which is supported by coprological investigations. In feces of dogs maintained on sufficiently rich carbohydrate rations there are observed many undigested starch granules.

Further experiments were directed toward clarification of the mechanism of increase in digestive juice amylase immediately after ligation. The question was raised whether or not the increase is connected with active production of the enzyme by intestinal glands. It is known that the secretion of enzymes in the intestines is regulated by the neural system. It was shown that denervation of an intestinal loop results in drastic decrease in production of a number of its enzymes [2]. In relation to amylase such investigations have not been conducted.

Intestinal denervation was performed by two methods. In two dogs one of the intestinal loops was isolated by Thiry's method, and the other was denervated by cutting the branches of mesenteric nerves; in the third dog one of the intestinal loops was isolated by Thiry's method, and the other was denervated by transplanting it under the skin and subsequently cutting the vascular-neural bundle. The starting secretory level was determined for both loops.

In agreement with previous literature [2], it was shown that the enterokinase and phosphatase content in juice from the denervated loop was sharply decreased. Denervation had no noticeable influence on the amylase content. The amylase concentration in juice from the denervated intestinal loop was not decreased; it remained at the same level as the Thiry loop.

After ligation of the glandular ducts the amylase content of the secretions from the denervated loop as well as from the Thiry loop was sharply increased (Fig. 3). During the first few days after operation juice from the denervated loop contained less amylase than the juice from the Thiry loop. On the third and fourth day the content was increased and surpassed the amylase content of the Thiry loop.

Subsequently, the amylase concentration in juice from both loops gradually decreases and in one and one-half to two weeks it returns to the starting level and thereafter it decreases further. These changes are correlated with blood amylase content.

Separate determinations of amylase in the solid and liquid portions of the juice, after ligation of the ducts,

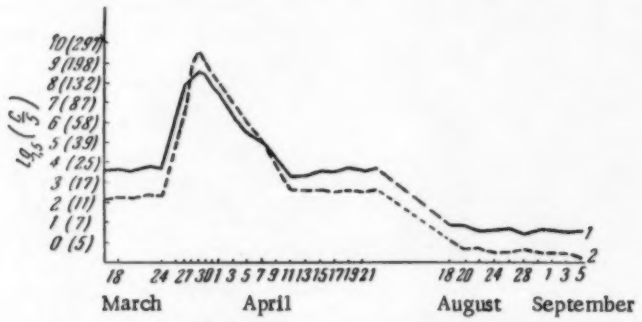


Fig. 3. Amylase production in the liquid portion of intestinal juice. 1) Isolated intestinal loop (Thiry); 2) Transplanted intestinal loop. The arrow ( ↓ ) points to the time of duct ligation and sectioning of the vascular-neural bundle of the loop.

In order to clarify the role of the neural system in the development of the above mentioned change in amylase production following ligation of pancreatic ducts, experiments were conducted with the denervated intestinal loop. In these experiments the same dog served as the source of intestinal juice from isolated Thiry loop and from a denervated intestinal loop. The humoral medium of both of the loops was the same.

have shown that with the sharp increase of amylase, the relative contents of amylase in both portions remained the same as prior to ligation, i.e., approximately in the same quantities or in the solid portion one and one-half to two fold greater than in the liquid portion. The same relationships are observed in the secretions from the denervated loop.

Thus the deficiency of amylase in the digestive tract

resulting from exclusion of exocrine pancreatic secretion does not lead to active production of this enzyme by intestinal glands, and compensatory increase of amylase does not occur (even in animals kept on a common, carbohydrate rich ration).

The increased output of amylase in intestinal juice after pancreatic duct ligation does not depend on the increase in its secretions from the intestinal glands.

The data showing the same amylase content in the liquid and solid juice portions, correlat.ng changes in the blood and intestinal juice after duct ligation, and likewise the absence of changes after denervation, allow the conclusion to be made that in dogs, after exclusion of the exocrine pancreatic secretions, the increase in amylase concentration of the intestinal juice depends on the transfer of this enzyme from the blood into the intestinal secretions.

It may be supposed that in the dog under normal conditions, the small amount of amylase found in the intestinal juice is similarly dependent on its transfer from the blood.

## SUMMARY

Both pancreatic ducts were ligated in dogs with the isolated (according to Thiery) and denervated intestinal loop. The following was established: The presence of almost the same amylase content in the fluid and thick portions of the intestinal juice, its correlating changes in the blood and intestinal juice after ligation of the ducts, the absence any changes of its content in denervation by way of section of the mesenteric nerves. A suggestion is made that the presence of amylase in the dog's intestine depends mainly upon the fact that this ferment is conveyed from the blood into the intestinal secretion. The intestinal enzymes enterokynase, alkaline phosphatase, lipase, peptidase and saccharase were also investigated.

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# THE ROLE OF THE INITIAL ARTERIAL PRESSURE IN THE DEVELOPMENT OF HEMORRHAGIC SHOCK

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An experimental model which has been used for the investigation of hemorrhagic shock consists of rats exposed to sound stimulation [3]. It was shown in this investigation that, with increased excitation of the nervous system (caffeine, parathyroidectomy), there was a considerable increase in the mortality rate from cerebral hemorrhage as the result of exposure to sound. The thyroid gland hormone had a particularly marked influence on the development of hemorrhagic shock [4]. The preliminary administration of thyroidin to animals increased the number of fatal cases after traumatization of the nervous system with a sound stimulus [4].

Further investigations [9] showed that, as the result of transient action (for up to 90 sec) of a sound stimulus, rats showed a considerable fall in the arterial pressure, a slight fall in body temperature and an increase in the blood homo globin. These changes were more marked in rats receiving thyroidin. After the prolonged action of a sound stimulus (up to 20 min) disorders of movement, areflexia and nystagmus were observed. This whole complex of symptoms and signs suggest that, as a result of trauma of the nervous system, a shock-like state develops, against which background cerebral hemorrhage may develop from the action of the sound stimulus and death of some of the animals may ensue.

We observed that the animals which died were more often those with a higher initial arterial pressure. The difference between the initial values of the arterial pressure in the animals dying as a result of the action of the sound stimulus and in those which survived was statistically significant ($R = 3.8$). We know from reports in the literature that the overwhelming majority of cerebral hemorrhages are the consequence of hypertension [5, 6, 8].

The aim of the present research was to study the role of the initial arterial pressure in the development of states of hemmorrhagic shock as a result of nervous trauma.

## METHOD

Experiments were carried out on 206 adult (from 3 to 6 months old) white laboratory rats, mainly males, sensitive to the action of a sound stimulus.

The animals were subdivided into two groups, one of which took part in the experiment in a normal state, and the other was given preliminary treatment with thyroidin.

The thyroidin was given orally through a glass tube daily in a dose of 25-50 mg for ten days. In order to produce hemorrhagic shock in the rats a method developed in our laboratory [3] was used. The method consists essentially of the interrupted action of a sound stimulus on the rat for 15 minutes, followed by a three-minute interval, after which the sound stimulus is applied for 90 seconds. The resulting excitation is very intensive and in several cases terminates by death of the animals from cerebral hemorrhage. Before the beginning of the experiment and

TABLE 1. State of the Arterial Pressure in the Animals Dying and Surviving as the Result of Nervous Trauma

| Group of animals | Surviving | | | | Dying | | | |
|---|---|---|---|---|---|---|---|---|
| | number of animals | arterial pressure (mean) | | | number of animals | arterial pressure (mean) | | |
| | | before expt. | after expt. | difference before and after expt. | | before expt. | after expt. | difference before and after expt. |
| Animals receiving thyroidin | 46 | 118 | 89 | —29 | 12 | 136 | 102 | —34 |
| Normal animals | 51 | 116 | 94 | —22 | 1 | 124 | 90 | —34 |

TABLE 2. Relationship between the Fall in Arterial Pressure and its Initial Value and the Intensity of the Animal's Response Reaction to the Sound Stimulus

| Initial arterial pressure below 120 mm Hg | | | | | Initial arterial pressure above 120 mm Hg | | | | |
|---|---|---|---|---|---|---|---|---|---|
| character of the response reaction of the sound stimulus | number of animals | arterial pressure (mean) before expt. | arterial pressure (mean) after expt. | diff. before & after expt. | character of the response reaction of the sound stimulus | number of animals | arterial pressure (mean) before expt. | arterial pressure (mean) after expt. | diff. before & after expt. |
| Motor excitation with fits . . . . . | 66 | 105 | 83 | —22 | Motor excitation with fits . . . . . | 8 | 126 | 81 | —45 |
| Motor excitation without fits . . . | 17 | 105 | 94 | —11 | Motor excitation without fits . . . . . | 5 | 127 | 95 | —32 |

after its conclusion the arterial pressure was determined by the method of Sidney and Friedman [11].

## RESULTS

By comparison with the survivors, the initial arterial pressure of the animals which died was higher, and a somewhat greater fall in its value was observed as a result of the nervous trauma (Table 1).

In order to elucidate the relationship between the arterial pressure fall and its intial value, we subdivided the ordinary experimental animals in accordance with the value of their initial arterial pressure into two groups. In one of the groups we included the animals with a pressure not exceeding 120 mm Hg, and in the other, animals in which the pressure was over 120 mm Hg. Both groups of animals were subjected to the action of continuous sound stimulus for 90 seconds.

It will be seen from the results in Table 2 that the fall in arterial pressure as a result of brief exposure to the sound depended on the magnitude of the initial pressure and also on the intensity of the animal's response reaction to the sound stimulus. The level of the arterial pressure after nervous trauma did not depend on the initial pressure, but it did depend on the intensity of the animal's response reaction to the sound stimulus.

What is the role of a high initial arterial pressure in the development of cerebral hemorrhages during the action of a sound stimulus? As a result of the action of the sound stimulus a sharp fall in the blood pressure of the animals took place, and this was more marked in those which died. Several authors have observed spasm of the cerebral vessels and a raised arterial pressure in animals at the moment of excitation, after which a sharp fall in the pressure and dilatation of the cerebral vessels ensued. L. A. Koreisha [2], for instance, points out that over-stimulation of the central nervous system during operation may cause an increase in the arterial pressure, accompanied by a sudden loss of consciousness and, occasionally, by epileptiform convulsions. The increase in the arterial pressure is quickly replaced by a catastrophic fall, with a sharp rise in the pulse rate. In the period of increased arterial pressure severe spasm of the vessels is observed, after which dilatation of both the arterial and venous systems ensues. These disturbances of neurodynamics, as this author observes, lead via dysfunction of the cardiovascular system to the escape of blood through the intact walls of the blood vessels.

On the basis of data in the literature we are inclined to consider that the sound stimulus causes spasm of the cerebral vessels in the animals, which then gives way to dilatation of these vessels and to a sharp fall in the arterial pressure. A high initial pressure is known to facilitate the onset of spasm [1, 2, 5].

The fall in the arterial pressure may thus act as an index of the functional changes taking place in the cerebral vessels. The level of the arterial pressure evidently cannot always characterize the severity of the animal's condition (see Table 2). Cases of severe shock with a comparatively normal pressure are described in the literature [10].

During morphological investigations [7] of the cerebral vessels in rats subjected to the action of a sound stimulus, dilatation of these vessels and hemorrhages into the brain were observed.

## SUMMARY

As a result of the action of a sound stimulus there occurs a sharp reduction of the arterial blood pressure in rats, some of which perished from cerebral hemorrhages. Animals with a higher initial value of the arterial blood pressure show a greater percentage of deaths. The results of this experiment demonstrate the relation between the degree of arterial blood pressure drop and the value of the initial blood pressure.

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# THE STATE OF THE RECEPTORS OF THE HINDLIMB IN DOGS DURING THE DEVELOPMENT OF A COLLATERAL CIRCULATION AND OF RADIATION SICKNESS

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Every operation is accompanied by division and ligation of blood vessels, which results in a change in the direction of the blood flow in certain areas of the body.

The course of development of collaterals in various organs and parts of the body has been established by experimental research and also by clinical observation.

The problem of the surgical treatment of injuries of various organs in irradiated subjects is indissolubly connected with the development of a collateral circulation.

Our investigations [4, 5], at first carried out in rats and subsequently in dogs, showed that the development of collaterals in the skin, muscles and nerves of irradiated animals takes place no less satisfactorily than in control animals observed after the same intervals of time. It was concluded that the plasticity of the blood vessels in animals suffering from radiation sickness evidently remains at a level capable of ensuring the development of a collateral circulation. Similar findings were obtained [1] during operations on the main blood vessels (circular suture, venous autografting) in dogs suffering from radiation sickness.

It is clear that the action of ionizing radiation is a factor mobilizing the potential resources of the blood vessels of the body. Under these circumstances new conditions are, of course, created for the structural and functional adaptation of collaterals.

The development of a collateral circulation in irradiated animals is thus an anatomo-physiological problem and requires complex investigations for its solution. Until recently the development of collaterals in irradiated animals has not been studied in connection with any definite function of the corresponding organ. Meanwhile it has been established in physiological experiments by D. M. Gzgzyan [2] that the afferent impulses from the limb in irradiated dogs change in accordance with the time elapsing after irradiation, i.e. with the period of development of radiation sickness.

We investigated the functional state of the receptor apparatus of the hindlimb in irradiated dogs during the development of a collateral circulation. In this way we attempted to examine the possible mechanism of action of ionizing radiation on the development of collaterals.

## METHODS

Experiments were carried out on 32 dogs. In the course of one day the animals were subjected to whole-body irradiation in a dose of 450 r (conditions: 185 kv, 15 ma, 0.5 mm Cu) and to the operation of resection of the femoral artery in one (the right or left) limb; the other limb usually served for comparison and control purposes.

Research by I. D. Lev [3] showed that, after resection of the femoral artery in dogs, collaterals develop mainly in the posterior group of thigh muscles, receiving blood from the hypogastric artery through branches of the caudal gluteal artery (Fig. 1). The anatomical basis of this fact is that the hindlimb of the dog, in contrast for example to the lower limb in man, is supplied with blood from two systems of arteries: the femoral and caudal gluteal arteries.

We therefore studied the afferent impulsation from the receptors of both hindlimbs after various intervals of time by the method of perfusion through the caudal gluteal arter, for the femoral artery in one of the limbs was removed during operation. Under chloralose or urethane anesthesia, both hindlimbs of a dog were separated from the trunk. The muscles were divided near their origins, together with the femoral and obturator nerves, and careful hemostasis was observed. The femoral artery of the unoperated limb was ligated and divided between two ligatures before the beginning of perfusion. The femur was disarticulated at the hip joint. The limbs were left connected to the trunk only by the sciatic nerve.

Both limbs were perfused through cannulas inserted into the caudal gluteal artery with Tyrode solution, enriched with oxygen and warmed to 38-39°. The perfusate flowed out through the femoral veins. Both limbs were thus kept under identical experimental conditions, so that a comparative study could be made of the afferent impulsation from their receptors. We may mention that in one limb (in which the femoral artery had been divided) the development of a collateral circulation had already reached a certain level, whereas the blood vessels of the other limb had not undergone any preliminary changes. We thus expected to find differences in the reflexes from the receptors in the two limbs under examination.

The presence of an afferent impulsation was judged by reflex changes in the arterial pressure and respiration arising after the injection of chemical stimulants into the blood stream of the limbs. As stimuli of the chemoreceptors we used a 2% solution of KCl and a 1 : 1000 solution of nicotine. These substances were injected into the per-

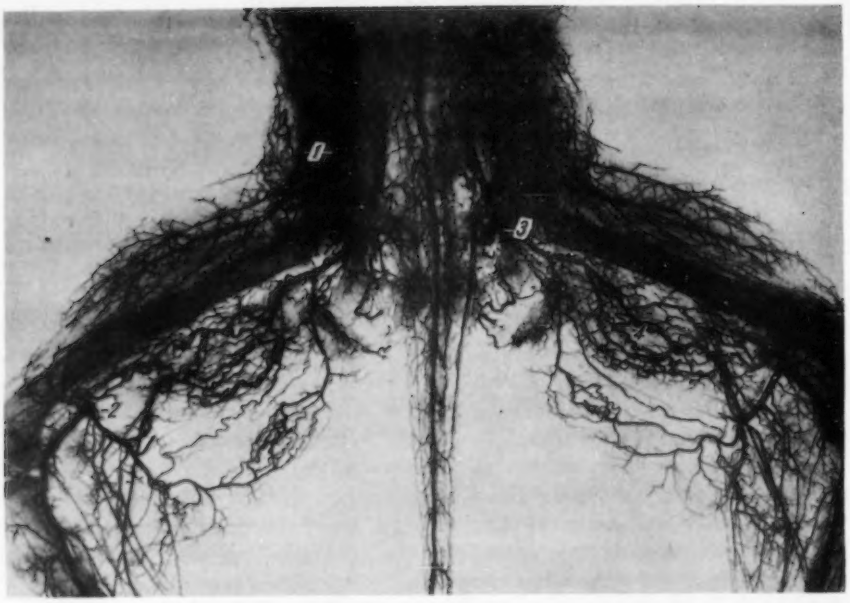

Fig. 1. Development of collaterals in the hindlimbs of a dog after resection of the femoral arteries (roentgenogram). 1, 2) Proximal and distal cut ends of the femoral artery; 3) caudal gluteal artery; 4) collaterals developing from the anastomoses of the caudal gluteal system of arteries with the popliteal artery.

fusate, directly into the cannula inserted into the caudal gluteal artery.

The arterial pressure was recorded with a mercury manometer in the left carotid artery. Respiration was recorded by means of a Marey's capsule, communicating with a corrugated tube bound around the animal's thorax. These indices, together with the zero line, the marker of injection of the drugs and the time marker were recorded on the kymograph.

## RESULTS

After whole-body irradiation with a dose of 450 r, the dogs began to develop acute radiation sickness. The animals' condition gradually deteriorated, and by the 11th-12th day after irradiation, they could not usually withstand the acute experimental conditions, which had to be limited to these times. In all, three series of experiments were carried out: at intervals of 4-6 days, 8-9 days and 11-12 days after irradiation. The blood was examined in all the dogs before the experiment (white cell count, ESR, hemoglobin).

On the 4th-6th day after operation and whole-body irradiation of the dogs, the changes observed in the afferent impulsation from the limbs, operated and unoperated, were not always in the same direction. In some animals there was a greater increase in the reaction from the receptors of the limb in which collaterals were developing. In these cases the receptors of the operated limb reacted to all the doses of the stimulus that were used, whereas the receptors of the other (control) limb did not respond to ordinary (2 ml of a 2% solution of KCl) doses; a perceptible afferent impulsation was elicited only by the use of double doses of the stimulus. In other animals of the same series of experiments, on the other hand, the afferent impulsation from the control limb was more pronounced than that from the operated limb. It must be assumed that, during this (latent) period of radiation sickness, changes were observed in the afferent impulsation to the central nervous system, which interfered with the regulatory activity of the latter.

At this time the collaterals of the caudal gluteal arterial system in the operated limb could still not be detected by anatomical methods, and hardly differed from the usual anastomoses of the arteries of this system in normal animals (i.e., in the control limb).

In the next series of experiments, 8-9 days after whole-body irradiation of the dogs, it was clearly seen that the receptors of the operated limb reacted more strongly to the injection of the stimuli into the perfusion fluid than did the receptors of the control limb (Fig. 2,a). This was particularly demonstrable after the injection of small doeses of the stimulus (1 ml of a 2% solution of KCl), which evoked more pronounced reflexes from the operated limb than from the control (Fig. 2, b).

It was apparent that the reception from the operated limb, in which collaterals were continuing to develop, was more increased than that from the opposite limb, in which collaterals were continuing to develop, was more increased than that from the opposite limb, in which the system of anastomoses of the caudal gluteal artery began

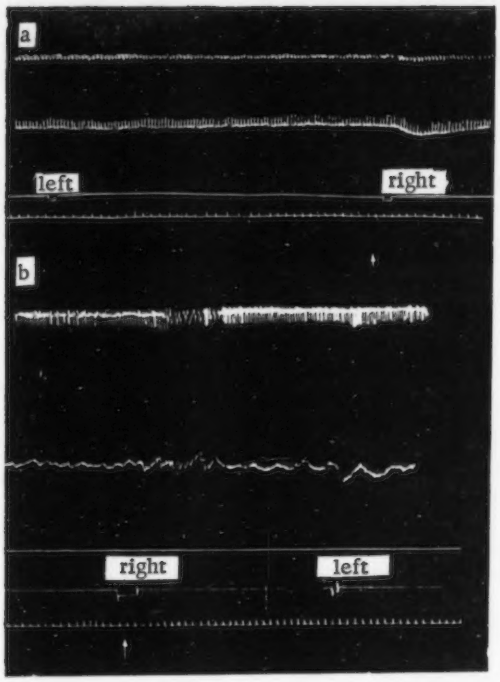


Fig. 2. Changes in the arterial pressure and respiration on the 8th-9th day after resection of the femoral artery ( ) and whole-body irradiation in dogs. Significance of the curves (from above down): Recording of respiratory movements; recording of arterial pressure; marker of injection of stimulus and zero line of arterial pressure; time marker (5 sec). a) After injection of 2 ml of 2% KCl; b) after injection of 1 ml of 2% KCl.

to function intensively only during the time of the acute experiment.

The results of this series of experiments confirmed the fact, obtained by anatomical methods of investigation [4, 5], that the plasticity of the blood vessels, without which the development of collateral channels of circulation cannot be contemplated, is evidently maintained at an adequately high level even during the conditions of acute radiation sickness. The blood analyses showed that the white cell count in the peripheral blood of these animals fell to 3000-850/ $mm^3$ (in one experiment 750), and the ESR reached 18-43 mm in 1 hour.

In the third series of experiments the dogs were included which survived 11-12 days after whole-body irradiation, when the period of crisis of radiation sickness was beginning. The white cell count fell to 600-350/ $mm^3$. The experiments on two dogs were performed at an arterial pressure of 70 and 35-40 mm Hg. The animals' condition had deteriorated to such an extent that some of them could not withstand the acute experimental conditions, but died soon after separation of the hindlimbs from the trunk, with a sharp fall in the arterial pressure.

In none of these experiments was it possible to obtain appreciable changes in the level of the arterial pressure and in the respiratory movements after the injection of any dose of the stimulus whatever (up to 20 ml of a 2% of KCl solution) into the artery on either the operated or the control limbs. At this time, meanwhile, as anotomical investigations showed (see Fig. 1), the development of collaterals continued. It must be assumed that the severity of the radiation sickness had a profoundly destructive effect on the function of the receptors, from which the afferent impulsation was sharply reduced under these conditions.

The results of the three series of experiments which have been described suggest that, during the development of a collateral circulation in irradiated dogs, changes take place in the afferent impulsation from the receptors of the limbs, as shown by: the lability of the reflexes, which is observed during the first 4-6 days of development of the collaterals and the radiation sickness; the increase in the reflexes, which corresponds to the further development of the collateral channels; and the depression of the reflexes commencing on the 11th-12th day of the acute radiation sickness while the process of collateral development continues.

A common tendency of the first two phases is the increase in the strength of afferent impulsation as the collaterals develop in the limb.

At the height of the disease a lack of correlation developed between the degree of formation of collaterals in the limb and the functional state of its receptor apparatus. What was the possible reason for this phenomenon? Where did the inhibition of the impulses take place: at the periphery, in the limb itself or in the central nervous system?

We felt that an attempt could be made to answer these questions by carrying out special experiments on dogs in which the resection of the femoral artery was performed simultaneously on both hindlimbs, and only one limb was exposed to x-ray irradiation in a dose of 450 r. The other limb thus acted as a control for comparison.

Eleven days after operation and local irradiation, changes could be observed in the reactions to the injection of ordinary doses of the chemical stimulus only from the unirradiated limb. Only when the dose of KCl was 5 ml were changes seen in the arterial pressure level and the respiratory movements from the irradiated limb.

The results of these experiments may be interpreted as showing the direct influence of ionizing radiation on the receptor apparatus of the limb. A further check had to be made of the hypothesis that depression of the central ends of the analyzers arises in the dogs on the 11th-12th day after whole-body irradiation.

For this purpose, in dogs exposed to whole-body irradiation and to unilateral operation on the limbs (as in the principal series of experiments), the sciatic nerve was stimulated after 11-12 days by means of an induction current (voltage 6 v, distance between coils 20 cm),

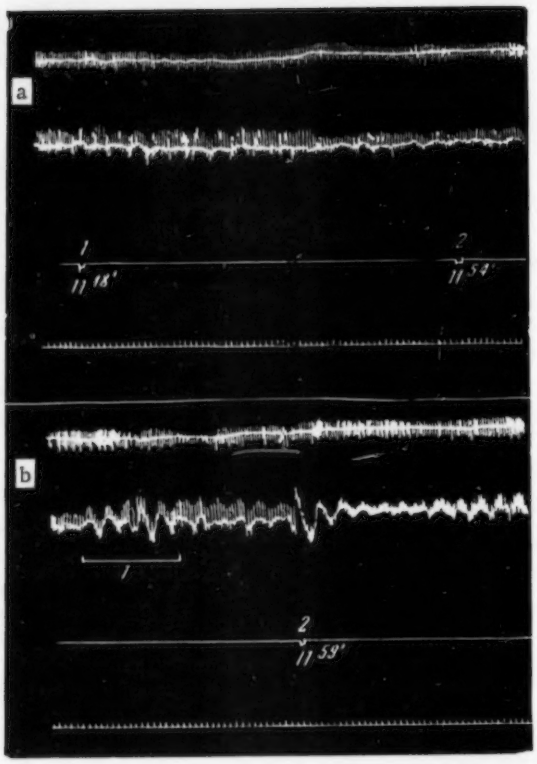


Fig. 3. Changes in the arterial pressure on the 11th day after resection of the femoral arteries and local irradiation of one hindlimb. a) Recording of respiration and arterial pressure after injection of 2% KCl: 1) 2 ml; 2) 3 ml; b) the same after insertion of electrodes and stimulation of the sciatic nerve with an electric current (2).

for no changes in the reflex reactions to chemical stimulation from the receptors of the limb could be obtained (Fig. 3,a). By the mere placing of the electrodes under the nerve, not to mention the time of actual passage of the current, a sharp increase took place in the amplitude of the cardiac contractions, with a fall in the arterial arterial pressure and a slowing of respiration (Fig. 3, b). These experiments showed that whole-body irradiation of an animal is accompanied both by disturbances of the function of the central nervous system and by direct change in the reflex apparatus of the organs and tissues of the limb

Our experiments provide new proof of the fact that the function of an organ depends not only on the number and diameter of the arteries supplying it, but also on the state of its nervous apparatus. Whereas an anatomical study of the development of collaterals during radiation sickness shows that their structural adaptation remains at a high enough level for the formation of a collateral circulation, their functional adaptation does not always proceed along the same lines, but changes in accordance with the severity (or stage) of the radiation sickness. So far as the causes of these changes are concerned, besides the local influence of inoizing radiation on the receptor apparatus, demonstrated in our experiments, it cannot be denied that central inhibition of impulses also arises under these conditions. The combination of both phenomena creates the complex picture of the mechanism of the reflex activity of the animal in acute radiation sickness.

## SUMMARY

The authors studied the effect of irradiation (450 r) on the condition of the receptors of the posterior limb of the dog in which a collateral circulation developed after resection of the femoral artery. The change of the afferent impulsation in the operated limb was shown by: a) Thelability of the reflexes observed during the first 4-6 days of development of collaterals in radiation sickness; b) the increase of the reflexes corresponding to the further development of the collateral circulation, and , c) depression of the reflexes beginning with the 11th-12th day from the onset of acute radiation sickness with the continuation of the process of development of collaterals.

The authors came to the conclusion that the structural adaptation of collaterals was adequate for the development of collateral circulation but its functional adaptation did not follow a parallel course and changed in accordance with the stage of radiation sickness.

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# THE EFFECT OF LIPOCAIC ON THE CHOLINERGIC REACTION OF THE BLOOD IN DOGS UNDERGOING PARTIAL PANCREATECTOMY

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It has been shown by A. V. Kibyakov and his coworkers [3, 4] that partial extirpation of the pancreas leads to a temporary disturbance of acetylcholine synthesis in animals. The mechanism of this phenomenon has not received adequate study.

According to investigations by many workers, partial depancreatization causes no significant changes in the level of the blood sugar [2, 3, 12, 17], whereas the blood lipid concentration falls appreciably [16]. Total depancreatization is always accompanied by the development of fatty infiltration of the liver, which is not removed by insulin [13, 15], but which is prevented by the influence of the lipotropic factor of the pancreas [5, 13, 15]. On the basis of the many investigations by S. M. Leites and his coworkers [5, 7, 8], this active principle of the pancreas is its second hormone, lipocaic. This hormone activates oxidation of the fatty acids in the liver and the formation of phospholipids [6, 9, 10]. Phospholipid metabolism is associated with the synthesis of acetylcholine.

We conceived the idea that the cause of the disturbance of acetylcholine synthesis during partial depancreatization of animals is a deficiency of the hormone lipocaic. In order to verify this hypothesis we investigated the influence of lipocaic on the formation of acetylcholine in animals subjected to a preliminary partial pancreatectomy. The index selected of the acetylcholine metabolism was the blood level of acetylcholine in experimental animals and the serum cholinesterase activity.

## METHOD

Experiments were carried out on dogs. From the saphenous vein, 5 ml of blood was drawn into a syringe containing 5 ml of eserine solution in a concentration of 1 : 50,000. The blood was defibrinated and diluted with Ringer's solution in a ratio of 1 : 10. The total dilution of the blood was thus 1 : 20. The acetylcholine concentration in the blood was estimated by Corsten's method [14] in the isolated frog's lung, in which contractions develop at an acetylcholine concentration of up to $1 \cdot 10^{-20}$. The exised lung of a frog was washed free from blood and kept in a solution of eserine (1 : 10,000) for three hours in the cold. The lung was then fixed in a small glass vessel (10 ml in volume), which was constantly perfused with Ringer's solution. The contractions of the lung were recorded on a kymograph. The blood for testing, and acetylcholine in different dilutions, was added to the fluid in the glass vessel in a volume of 1 ml. After each test the lung was thoroughly washed until complete relaxation was achieved. At the beginning and end of the experiment the degree of sensitivity of the lung to acetylcholine was tested. The acetylcholine concentration in the blood for testing was determined by means of the concentration of acetylcholine solution causing a contraction of the lung of equal height on the kymograph, with a subsequent calculation for whole blood.

The serum cholinesterase activity was estimated by a titration method, as described by T. V. Pravdich-Neminskaya [11]. The method is based on the decomposition of acetylcholine by the cholinesterase of the test serum with the formation of choline chloride and an equivalent quantity of acetic acid. The acetic acid was titrated with a 0.01 N solution of NaOH in the presence of a 0.02% solution of cresol red as an indicator. The titration was carried out from a microburette of 0.2 ml capacity and with divisions of 0.002 ml. The percentage of acetylcholine decomposed in unit time was taken as a measure of the activity of the enzyme.

The investigations described were carried out in the first place on unoperated dogs, and later at various periods after the operative removal of part (3/5) of the pancreas, with simultaneous ligation of its ducts. Daily observations were made on the animals until 30-38 days after operation. Of the six dogs used in the experiment, three were controls and did not receive lipocaic in the postoperative period; starting on the first day after the operation, the other three dogs received five tablets of lipocaic, i.e., 50 units, daily with their food. All the dogs were of roughly the same weight and were kept on a normal diet (meat, bread and meal). The experiments were performed at the same time of day. The animals were kept under the same conditions during the pre- and postoperative periods.

## RESULTS

Before partial extirpation of the pancreas, the acetylcholine concentration in the blood of all the experimental dogs was $2 \cdot 10^{-6}$–$2 \cdot 10^{-4}$, and the serum cholinesterase

activity fluctuated between 29.6 and 19.1%. The blood acetylcholine level and the degree of cholinesterase activity on different days varied within very narrow limits in each animal.

After partial pancreatectomy a considerable fall in the blood acetylcholine level was observed in the three dogs which did not receive lipocaic. This fall was apparent after the second postoperative day and reached its maximum on the sixth day after operation. The concentration of acetylcholine in the blood was only $2 \cdot 10^{-15} - 2 \cdot 10^{-14}$. Later, the acetylcholine level began to rise, but on the 8th-14th day it was still low ($2 \cdot 10^{-12} - 2 \cdot 10^{-9}$ Even on the 38th day after operation the acetylcholine concentration in the blood had still not reached fully its preoperative level (acetylcholine was determined in the blood in concentrations of $2 \cdot 10^{-8} - 2 \cdot 10^{-7}$). At the same time as the fall in the acetylcholine concentration, a fall in the serum cholinesterase activity was also observed. The maximum fall was observed on the sixth postoperative day (3.6-9.1%) and was followed by a gradual rise, but on the 38th day after operation the cholinesterase activity had not reached its initial values. The serum cholinesterase activity varied between 15.4 and 18.3% at this period after operation (the 38th day).

In the three depancreatized dogs which received lipocaic in the postoperative period, the pattern of the changes in the cholinergic reaction of the blood was different. In the first days after operation a fall in the blood acetylcholine level was observed, and this was especially pronounced on the sixth day after operation; the degree of this fall, however, was much less than that observed in the control animals. The blood acetylcholine level was $2 \cdot 10^{-11} - 2 \cdot 10^{-5}$. In the subsequent postoperative days the blood acetylcholine concentration rose rapidly. Whereas on the eighth day after operation the blood acetylcholine level was still low ($2 \cdot 10^{-8} - 2 \cdot 10^{-5}$) by comparison with the normal values for these animals, on the 14th day after operation the blood acetylcholine had fully regained its initial values ($2 \cdot 10^{-4} - 2 \cdot 10^{-6}$) in two of the experimental dogs, and in only one dog was this restoration of the initial level delayed until the 18th day after operation. On all the following days of observation (until the 30th-38th postoperative day) the blood acetylcholine level was maintained within normal limits (preoperative period). The serum cholinesterase activity in these dogs on the 6th-8th day was slightly lowered (17.2-11%), and on the 14th day it had reached normal in one dog and nearly normal in the other two dogs. On the subsequent postoperative days the serum cholinesterase activity was identical with its value in the preoperative period. The course of the changes in the blood acetylcholine level and in the serum cholinesterase activity in the individual experimental dogs in the postoperative period is demonstrated by the figures in the table.

In the animals receiving the hormone lipocaic after partial pancreatectomy, the weakening of the cholinergic reaction of the blood was thus less prolonged and much less pronounced than in the control animals. In our experiments, side by side with the fall in the acetylcholine level, a fall in the serum cholinesterase activity was ob-

| Animals | Concentration of acetylcholine in the blood | | | | | Serum cholinesterase activity (in %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | before operation | after operation | | | | before operation | after operation | | | |
| | | 6th day | 8th day | 14th day | 38th day | | 6th day | 8th day | 14th day | 38th day |
| Dogs subjected to partial depancreatization | | | | | | | | | | |
| Santa | $2 \cdot 10^{-5}$ | $2 \cdot 10^{-15}$ | $2 \cdot 10^{-11}$ | $2 \cdot 10^{-9}$ | $2 \cdot 10^{-7}$ | 21 | 3,6 | 10,9 | 15,4 | 15,4 |
| Chernyi | $2 \cdot 10^{-5}$ | — | $2 \cdot 10^{-10}$ | $2 \cdot 10^{-10}$ | $2 \cdot 10^{-8}$ | 20,9 | — | 11,0 | 18,1 | 18,3 |
| Barbos | $2 \cdot 10^{-6}$ | $2 \cdot 10^{-14}$ | $2 \cdot 10^{-12}$ | $2 \cdot 10^{-10}$ | $2 \cdot 10^{-8}$ | 20,0 | 9,1 | 13,6 | 17,2 | 18,85 |
| Dogs receiving lipocaic after depancreatization | | | | | | | | | | |
| Lokhmatyi | $2 \cdot 10^{-4}$ | $2 \cdot 10^{-5}$ | $2 \cdot 10^{-5}$ | $2 \cdot 10^{-4}$ | — | 29,6 | 17,2 | 16,8 | 26,0 | — |
| Kerri | $2 \cdot 10^{-6}$ | $2 \cdot 10^{-9}$ | $2 \cdot 10^{-8}$ | $2 \cdot 10^{-6}$ | $2 \cdot 10^{-6}$ | 19,1 | 16,4 | 16,0 | 19,1 | 19,2 |
| Ovra | $2 \cdot 10^{-6}$ | $2 \cdot 10^{-11}$ | $2 \cdot 10^{-8}$ | $2 \cdot 10^{-8}$ | $2 \cdot 10^{-6}$ | 21,0 | 11,0 | 13,6 | 16,4 | 20,2 |

Changes in the Concentration of Acetylcholine in the Blood and the Serum Cholinesterase Activity in Depancreatuzed Dogs not Receiving and Receiving Lipocaic

served. The cholinesterase present in the serum of dogs is known to be mainly nonspecific and its activity is maximal at relatively high concentrations of acetylcholine [1]. The decrease in the serum cholinesterase activity in the depancreatized dogs was thus, in itself, an index of the disturbance of acetylcholine formation. On the other hand, the increase in the serum cholinesterase activity in the depancreatized dogs receiving lipocaic was evidence of the normalization of the acetylcholine metabolism under the influence of this hormone.

## SUMMARY

From analysis of the results obtained it may be concluded that lipocaic is able to a considerable degree to prevent those disturbances of acetylcholine formation which as a rule are found at certain postoperative periods in depancreatized animals. The main cause of these disturbances is consequently a deficiency of this hormone. This suggests that the pancreatic hormone, lipocaic, plays a part in the control of the synthesis of acetylcholine, one of the most important mediators of the nervous system. The results which we obtained are of obvious interest in the evaluation of the action of lipocaic in clinical con-

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# THE STATE OF THE LIMB TISSUES AFTER TRAUMA TO THE SCIATIC NERVE (AS DEMONSTRATED BY VITAL STAINING)

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The problem of the nervous control of the physiological functions of the body and of its individual organs and systems has been generally applied by Soviet physiologists and pathologists in their teaching of the trophic influence of the nervous system and of the nervous dystrophies. The work of I. M. Sechenov [9], I. P. Pavolv [8], and V. M. Bekhterev [1] has revealed a number of important aspects of the trophic maintenance of the tissues.

This trophic influence of the nervous system, maintaining the normal state of the tissues, as A. D. Speranskii showed [10], is often revealed in its negative form, i.e., through processes of a neuro-dystrophic character. Various injuries to the nervous system, at the periphery or in the centers, are reflected in the tissues by a complex group of morphological, functional and metabolic disturbances, which at the same time bring into play the protective and compensatory reactions of the body. The analysis of these processes, their phasing in time and their localization in space are of great importance to physiology, pathology and clinical medicine. That is why, for many decades, there has been no end to the search for methods which would enable the discovery of the quantitative and qualitative characteristics of the development of neurodystrophic processes and the forms of their compensation.

When we studied the relevant material and sought means to ascertain the state of the tissues during life after certain types of injury to the nervous system, we directed our attention to the possibilities presented by the method of vital staining, as developed by D. N. Nasonov and V. Ya. Aleksandrov [6, 7] in connection with the study of the problem of paranecrosis. We know from the work of these authors that any change in the physicochemical state of the tissues or any slight reversible denaturation of the tissue proteins, caused by different stimuli, influence the sorptive power of the tissues in respect of vital dyes.

Having set out with aim of evaluating the changes arising in the tissues during the development of a nervous dystrophy, we used the relationship of the tissues to vital dyes as an index of their condition. For the purpose of our investigation, however, the method of vital staining had to be essentially modified and adapted to the study of the trend of the tissue changes in the intact animal.

## METHOD

The work was undertaken on white rats weighing 130-150 g. In order to reproduce a neurodystrophic process in the animals, the left sciatic nerve was divided in aseptic conditions in the upper third of the thigh. The central end of the nerve was treated with 0.02-0.05 ml of a 2% solution of formalin. After various intervals of time (3, 7, 14 days), the animals were given an intravenous injection (into the saphenous vein) of a 1% solution of neutral red in Ringer's solution (without $NaHCO_3$) in a dose of 0.1 ml per 30 g body weight, i.e., of 0.033 mg of the dry dye per g body weight.

In order to ascertain the degree of vital staining of the tissues, we used the method of elution of the dye, followed by colorimetry of the extracts. The dye was eluted with a 1% solution of hydrochloric acid in 96° ethyl alcohol. Elution of the dye from weighed samples of the respective tissues continued for 24 hours. The colorimetry was carried out by means of a FEK-M-3 photoelectric colorimeter. The quantity of dye was calculated per g weight of tissue and expressed in $\gamma$.

In order to determine the quantity of dye taken up by the tissues, the animals were decapitated one hour after intravenous injection of the dye. The animals in which the rate of excretion of neutral red from the tissues was determined were sacrificed $2\frac{1}{2}$ hr after injection of the dye.

The basis of the technique which we employed consequently was: a)The principle of vital staining of the tissues of the body in vivo, the vital dye reaching the tissues via the blood stream; b) the principle of studying not only the sorptive power of the tissues, but also their power of excreting the dye in the course of a given period of time.

This approach enabled us to carry out the experiment in natural conditions and prevented possible artefacts due to the staining of tissues in vitro. The method enabled us to study the relationships which held good between the processes of accumulation and excretion of dye, so that a more complete picture could be obtained of biophysical and biochemical state of the tissues.

## RESULTS

The degree of staining and the excretion of the dye by the muscles of the calf and thigh and also of the foot on the side of the injured nerve and on the contralateral side were studied in 50 white rats. In 30 animals the left sciatic nerve was divided and the central end of the nerve treated with formalin. In the animals of group 1 (15 rats) the content of vital dye in the tissues was determined one hour after its intravenous injection on the third, seventh and 14th days of development of the dystrophic process. In the animals of group 2 (15 rats), the content of dye was determined in the tissues $2\frac{1}{2}$ hr after injection at the same periods after trauma to the sciatic nerve. Five rats were investigated at each period. The remaining 20 rats acted as controls (ten for each group).

It can be seen from the details in Fig. 1 that on the third day after injury to the sciatic nerve a considerable accumulation of neutral red took place in the calf muscles of the injured limb, whereas in the opposite limb the level of accumulation of dye fell appreciably. Under these circumstances an increased content of dye was determined on the injured side until the 14th day. On the contralateral side at the 14th day the level of accumulation of dye by the calf muscles reached the value observed in the control animals. A different relationship was shown by the accumulation of dye by the thigh muscles. On the injured side no essential changes in accumulation were observed, whereas on the contralateral side the muscles also behaved like the calf muscles of the same side in respect of accumulation of dye (Fig. 2).

Since we wished to ascertain to what extent the power of accumulation of vital dye not by individual tissues but by their natural groups changed under these conditions, we selected as a suitable object the complete foot. It can be seen from Fig. 3 that on both the injured the contralateral sides a fall in the accumulation of dye by the tissues of the foot took place at all periods.

In the process of development of dystrophy after trauma to one sciatic nerve, regular changes in the accumulation of vital dye by the tissues of both limbs were thus observed. Under these conditions the changes on the injured side could take the form of an increase in the level of fixation of dye or a decrease in the degree of staining of the tissues, depending on the object of investigation. In the contralateral limb a fall in the degree of fixation of dye was observed in all cases, with a tendency for it to normalize on the 14th day of the experiment to the control level. In accordance with the views of D. N. Nasonov and V. Ya. Aleksandrov [5, 6, 7], the changes observed in the tissues were regarded by us as reactions of a paranecrotic character, associated with a reversible denaturation of the tissue proteins.

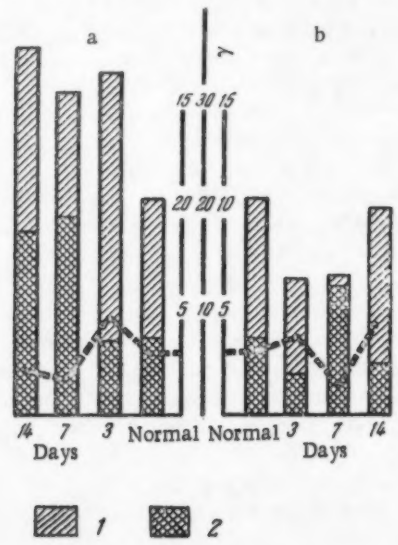


Fig. 1. Trend of the changes in vital staining of the calf muscles after trauma to the sciatic nerve. a) Left side (trauma to the sciatic nerve); b) right side. Conventional signs: Whole column (1, 2) – content of dye in the tissues one hour after its injection (sorption); lower part of the column (2) – content of dye in the tissues $2\frac{1}{2}$ hr after its injection (residue); broken line – ratio of sorption of dye to residue (trophic potential).

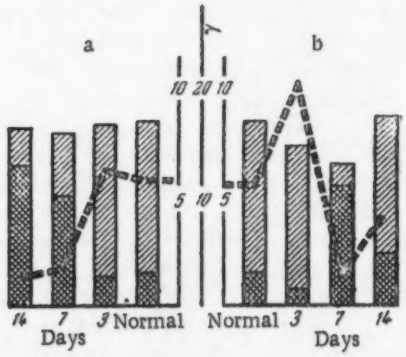


Fig. 2. Trend of the changes in vital staining of the thigh muscles after trauma to the sciatic nerve. a) Left side (trauma to the sciatic nerve); b) right side. Conventional signs as in Fig. 1.

Different variants of the paranecrotic reaction were observed in our experiments. A noteworthy fact in this case was the decrease in the fixation of dye by the tissues in the contralateral limb, which may be regarded as a

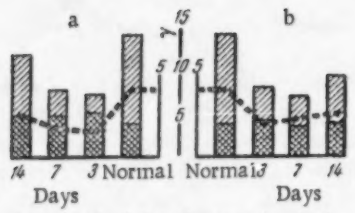


Fig. 3. Trend of the changes in vital staining of the foot after trauma to the sciatic nerve. a) Left side (trauma to the sciatic nerve); b) right side. Conventional signs as in Fig. 1.

manifestation of a compensatory reaction of the animal in response to pathogenic stimulation. The asymmetry of the reactions of the limb tissues indicated their reflex origin.

D. N. Nasonov and V. Ya. Aleksandrov [5, 6, 7], on the subject of changes of a similar type in the state of tissues, point out that in processes of denaturation of proteins, reactions of a compensatory character should be distinguished, for they tend in the opposite direction. These reactions also lie at the basis of reversible paranecrosis, and of the fact that this process is biphasic in its course. This biphasic character of the paranecrotic reactions enabled A. A. Ukhtomskii [12], D. N. Nasonov and V. Ya. Aleksandrov [6, 7], L. L. Vasil'ev [2], N. V. Golikov [4], and their coworkers to associate the reactions of paranecrosis with the reactions of parabiosis of N. E. Vvedenskii [3].

Analysis of the results obtained with regard to the problem of the relationship between the trophic state of the tissues and their reactions of a paranecrotic character showed that it was difficult by one index of the accumulation of vital dye by the tissues to judge the changes occurring therein. A quantitative estimation of the vital dye accumulating in the tissues tells nothing of the ability of the tissues to rid themselves of vital dye, which represents ballast to them. Meanwhile, the function of normalization of the tissues, indissolubly connected with the function of compensation of disturbances of function, is a more important aspect for consideration in the evaluation of their physiological state. Besides the work described above, we therefore carried out at the same time an investigation of the power of the tissues to excrete vital dye.

It is apparent from the figures shown that the power of the tissues of the injured and uninjured limbs to excrete vital dye did not follow a parallel course to their power to fix the dye. In some cases an increase in the fixation of neutral red was accompanied by its increased excretion (on the third day – the calf muscles of the injured limb), and in others the rates of excretion of the dye fell behind the rates of fixation. In these conditions different variants of the relationships between the values of the accumulation and excretion of dye were observed. A decrease in the degree of excretion of the dye might be observed: with an increased level of accumulation of dye (seventh day – calf muscles on the injured side); with a normal level of accumulation (seventh day – thigh muscles on the injured side) and with a simultaneous fall in the level of accumulation (seventh day – calf and thigh muscles in the uninjured limb). Finally, in some cases a fall in the level of accumulation of dye was accompanied by an increase in its excretion (third day – thigh muscles on the uninjured side).

The relationships thus revealed between the accumulation and excretion of vital dye by the tissues gave a more complete definition of the biophysical and biochemical state of the tissues. Advantage is taken here of the fact that the index of clearance of ballast dye from the tissues reflects the incorporation of compensatory mechanisms directed towards the normalization of their functional state.

In order to obtain a quantitative picture of the trophic potential of the tissues a coefficient was adopted which expressed the ratio between the amount of dye adsorbed and the amount remaining in the tissues. When determined in this way, the trophic potential of the tissues is a numerical index, not only of changes in the original resistance of the tissues in relation to corresponding stimuli, but also of their ability to restore and maintain their biophysical and biochemical state at the optimal level in accordance with the environmental conditions.

An analysis of the findings shows the value of this numerical index in the evaluation of the state of the tissues.

If, for example, consideration is taken only of the accumulation of neutral red by the thigh muscles on the side of the injured nerve, the conclusion may be reached that the muscles had undergone no changes, whereas, in fact, on the seventh and 14th days there had been sharp falls in the ability of these muscles to excrete the dye. The results obtained point to the prospects of analysis of the mutual relationships of nervous dystrophic processes, paranecrosis and processes of a parabiotic character.

All these processes ultimately reflect the changes undergone by the functional mobility or lability of the tissue substrates in normal and pathological conditions. In this connection we must remember the statement by A. A. Ukhtomskii, that: ". . . any tissue which more resiliently resists alteration more readily modifies it to suit its own ends and more rapidly returns to its original preparedness for work and will at the same time be more labile".

The definition of the trophic potential of a tissue by means of the paranecrotic test coincides fully with A. A. Ukhtomskii's statement regarding tissue lability. An increase in the coefficient of trophic potential coincided in our experiments with a more rapid clearance of dye from the tissue, and conversely, a decrease indicated a diminution of this power. Our test may thus be used to give a more profound insight into the condition of a tissue, which is of great importance in the analysis of a wide range of problems connected with nervous trophicity and nervous dystrophies and with the problem of compensation of physiological functional derangements in the body.

## SUMMARY

The authors modified the method of supravital staining of the tissues developed by D. N. Nasonov and V. Ya. Alexandrov. The staining was conducted in an intact animal by injecting neutral red into the blood stream. The study of the sorption of the stain and the process of its liberation by the tissues made it possible to put forward a definition of trophic potential of the tissues. Unilateral trauma of the sciatic nerve (section and treatment of its central end by a 2% formalin solution) asymmetrically changes the coordination between the acculation and liberation of the stain by the muscles of the shin and thigh, as well as by the tissues of the foot. This reveals phases characterized by the rise and fall of the trophic potential, pointing to the inclusion of the compensatory mechanisms paralleling the development of the neurodystrophic process.

The processes of paranecrosis, parabiosis, and those of a trophic condition of the tissues are compared theoretically.


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# SEQUELAE OF DECORTICATION IN DOGS WITH LATERAL HEMISECTION OF THE BRAIN STEM

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In our previous research [7, 8], we showed that lateral hemisection of the brain at the level of the pons varolii in dogs is accompanied by profound disturbances of the motor functions. After one or two weeks the dogs are unable to stand and walk, the neck and trunk are curved towards the side opposite the section, and the extensor tone in the ipsilateral limbs and the flexor tone in the contralateral limbs are strongly increased; involuntary automatic movements of the contralateral limbs are observed. The functions disturbed in consequence of hemisection are gradually restored in the course of 1-2 months to such an extent that the animals subjected to operation are externally indistinguishable from healthy animals; only a more detailed examination revealed certain motor disturbances. The subsequent removal of the cortex of the hemisphere opposite the hemisection causes repeated and profound disturbances of the motor functions in the dogs which were restored after the first operation. The functions disturbed a second time as a result of removal of the cortex of the hemisphere opposite the hemisection are again gradually restored (in the course of 1-1 ½ months), but not completely; even a long time after the second operation (as much as 7 ½ months) certain motor disturbances may still be observed, especially during the performance of complicated motor actions by the animals.

It could thus be postulated that the functions disturbed in consequence of decortication of one cerebral hemisphere are restored by means of the undamaged cortex of the second hemisphere. In order to investigate this problem more fully, in four dogs with hemisection of the brain stem at the level of the pons and subsequent unilateral decortication, we removed the cortex of the second cerebral hemisphere.

## METHOD

Experiments were carried out on four adult dogs in which, a few months before operation, a left-sided hemisection of the brain stem had been carried out at the level of the pons, followed by removal of the cortex of the right cerebral hemisphere. The cortex of the second, left, hemisphere was removed after all possible restoration of the functions impaired by the previous operation had taken place. In all cases the cerebral cortex was extirpated surgically.

## RESULTS

The experiments showed that removal of the cortex of the second hemisphere caused profound disturbances of the previously restored motor functions. The animals lost their ability to stand and walk, their movements became chaotic and incoordinated, and the dogs often adopted unnatural poses, tucked their head beneath them and thrust their right forepaw on their head. It could be seen that increased tone was present in the extensors of the limbs on the same side as the section, and in the flexors of those on the opposite side.

The dog Alpha, 6 ½ months after decortication of the second cerebral hemisphere. (Hemisection of the brain stem was performed on April 2, 1956, decortication of the first hemisphere on May 22, 1956 and of the second on September 24, 1956.)

In the postoperative period all the dogs displayed great restlessness, performed haphazard movements of all the limbs and struck the walls of the box in which they were kept with their head; it therefore became necessary to keep the animals in a sleeping condition by means of hypnotic drugs.

In the course of time some degree of restoration of the deranged functions of these animals after operation took place.

At the end of the first month, the dogs began to raise themselves on all four limbs. During this action it could be observed that the motor function of the hindlimbs was

disturbed to a far greater degree than that of the forelimbs. On standing, the animals' paws were placed widely apart, the head was placed low and the trunk often flexed laterally; the dogs quickly lost their balance and fell.

At the end of the second month, the dogs now rose from a recumbent position fairly quickly, but could only stand for a short time, and then lost their balance and fell. The gait was not regained at all by the dogs after removal of the cortex of both hemispheres and hemisection of the brain stem at the level of the pons varolii. In one dog, Malyshka, rare stepping movements were observed, but for the greater part of the time she stood immobile, with the head hanging down. Another dog, Alpha, sometimes took one or two steps backwards or moved the hindlimbs for a few steps in a circle to the right, thus turning around the forelimbs; the forelimbs under these circumstances also made stepping movements but a much slower rate than the hindlimbs. In a third dog, Bobik, flexion of the right hindlimb was extremely pronounced, so that the animal could hardly stand; no stepping movements by this dog were observed.

No subsequent improvement in the motor activity of the animals was observed, and the general picture for each animal a long time after the operation of removal of the cortex of the second hemisphere remained unchanged: the animals could not walk and their supporting functions remained grossly deranged (see figure).

After removal of the cortex of the second cerebral hemisphere, all the animals showed the presence of trophic ulcers, trophic changes in the skin accompanied by desquamation and falling out of the hair; scratches and abrasions constantly developed, caused by the animal's haphazard movements. It must also be mentioned that after bilateral decortication the dogs became extremely restless; an attempt to keep them in any one position caused severe motor restlessness, which prevented the more detailed examination of the dogs by the use of special methods.

Thus the removal of the cortex of the second cerebral hemisphere in dogs after preliminary lateral hemisection of the brain stem led to profound and prolonged disturbances of the locomotor and supporting functions; with the passage of time, the righting reflexes and the supporting functions were to some extent restored, as a result of which the animals became able to raise themselves from recumbency and to stand for a short time, although they could not walk.

According to the literature [1, 4, 6, 10, 11], on the second day after the two-stage removal of the cortex of both hemispheres, dogs can eat unaided, and on the 3rd-5th day they begin to walk; G. P. Zelenyi [4] even describes coordinated "play" movements in a decorticated young dog. In dogs decorticated after preliminary lateral hemisection of the brain stem at the level of the pons varolii, in contrast to decorticated dogs with no other injury to the central nervous system, the supporting and locomotor functions remained considerably disturbed and were only partially restored. On the other hand, in dogs with lateral hemisection of the spinal cord at different levels after bilateral decortication, standing and walking were not regained regained at all [1, 5].

The cerebral cortex thus plays an important part in the restoration of the functions disturbed in consequence of lateral hemisection of the brain stem at the level of the pons varolii; but the part it plays is smaller in this case than after lateral hemisection of the spinal cord. The divisions of the central nervous system lying below the level of section – such as the medulla oblongata and the lower part of the pons – evidently take part also in the compensation of the deranged functions.

The experimental data thus obtained on the participation of the cerebral cortex in the restoration of functions deranged by injury to the brain stem are in agreement with clinical observations. In the presence of pathological focus in the brain stem, the cerebral cortex undertakes, by means of the formation of new conditioned reflex connections, a complex and manifold reorganization of the functions of the nervous pathways and centers in compensation for the disturbed activity of the brain stem [2, 3, 9].

The findings obtained are also in agreement with E. A. Asratyan's main views on the leading role of the cerebral cortex in adult higher animals in the compensatory adaptations developing in the course of organic lesions of the nervous system.

## SUMMARY

Removal of the cortex of the second hemisphere in dogs after hemisection fof the brain stem at the level of the pons and subsequent unilateral decortication provoked deep disturbances of the reestablished motor functions. With time the righting reflexes and the locomotor functions in these animals showed a certain degree of recovery, while standing and walking functions still remained considerably disturbed. Thus in adult animals the cerebral cortex plays an important role in the restoration of the motor functions, deranged as the result of lateral hemisection of the brain stem at the level of pons varolii.

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*Original Russian pagination. See C. B. Translation.

# REFLEX CONTROL OF VASCULAR TONE IN ANAPHYLACTIC SHOCK

## Communication I. The State of the Pressor Reflexes During Hypotension Due to Anaphylactic Shock

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The study of the nervous reflex control of the vascular tone in a state of shock may help to explain the role of the nervous system in circulatory disorders and to find effective methods of dealing with the low arterial pressure in shock. In particular, an investigation of the pressor vascular reflexes will give some information on the value of certain methods of stimulation of afferent pressor mechanisms during the hypotension associated with shock.

There are few reports in the literature dealing with the reflex control of vascular tone in anaphylactic shocks. The only information [3, 6, 12] is of a considerable depression of the pressor vascular reflexes after the injection of an assaulting dose of antigen into the animal.

Many researchers (5,7-10] are inclined to believe that the state of shock, and the disorders which are then observed, are connected with the development of a parabiotic state in the central nervous system.

In view of this hypothesis, corresponding changes might have been expected to take place in the character of the vascular reflexes in anaphylactic shock.

The present investigation is devoted to the study of the pressor vascular reflexes after the injection of an assaulting dose of antigen into an animal, both at the onset of the fall in arterial pressure and during its recovery.

The capacity of the arterial pressure to be gradually, although slowly, restored, indicates that the experimental results considered below relate to a moderately severe state of shock.

## METHOD

Experiments were carried out on 32 rabbits and 6 dogs under nembutal anesthesia. The animals were sensitized by two subcutaneous injections of horse serum in a dose of 1 ml/ kg body weight for the rabbits and 0.5 ml/ kg body weight for the dogs. On the ocassion of the second injection of serum, after an interval of one day, the sensitizing dose was doubled. The assaulting dose of serum, which for the rabbits was 0.2 ml/ kg body weight, and for the dogs 0.25 ml/ kg body weight, was injected intravenously on the 15th-18th day of sensitization.

The arterial pressure was recorded in the common carotid artery by a mercury manometer on a smoked drum, and respiration by means of a Mary's capsule connected to a tracheotomy cannula. The pressor vascular reflexes were elicted by stimulation of the central end of the vagus (rabbit) or sciatic (dog) nerve with rectangular impulses from a generator, and also by acute asphyxia from closure of the tracheotomy tube.

## RESULTS

In normal conditions the average arterial pressure of the rabbits was 108 mm Hg. After the intravenous injection of the assaulting dose of serum, in nearly every case a transient increase in the arterial pressure was observed, followed by a fall to 40-50 mm Hg. The arterial pressure was then gradually restored, and after 35-40 minutes its level was 75-80 mm Hg.

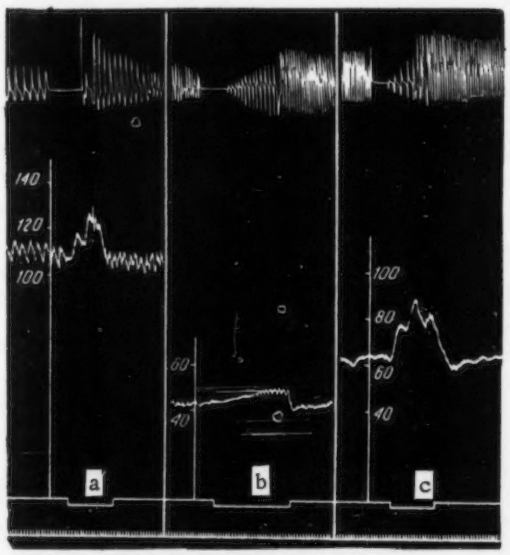


Fig. 1. Changes in the pressor vascular reflex during stimulation of the central end of the vagus nerve in the rabbit. a) Normal; b) 5 minutes after injection of the assaulting dose of serum; c) 30 minutes after. Significance of the curves (from above down): Respiration, arterial pressure; stimulation marker; time marker (1 second).

In the first minutes of the fall in the arterial pressure, the pressor reflexes caused by stimulation of the central end of the vagus nerve were considerably depressed. Whereas the magnitude of the normal pressor reflexes was on the average 14 mm Hg, immediately after the fall in pressure the magnitude of these reflexes did not exceed 10 mm Hg (Fig. 1, a, b). Subsequently, despite the lowered arterial pressure, stimulation of the central end of the vagus nerve was accompanied by an even greater pressor effect (Fig. 1, c). In the majority of experiments the magnitude of the pressor reflex was equal to that of the normal reflex, or even exceeded it, after only 20-30 minutes. This increase in the strength of the reflexes over that normally observed was especially obvious at the 35th-40th minute of lowered arterial pressure.

The same changes in the pressor reflexes were found also in experiments on dogs during stimulation of the central end of the sciatic nerve. It should be mentioned that although the injection of the assaulting dose of antigen was accompanied by a larger fall in pressure than during the experiments on the rabbits, the pressor reflexes were not merely restored but even strengthened by comparison with normal (Fig. 2). Moreover, in the control animals at the end of the period of stimulation of the sciatic nerve a depressor vascular reaction often appeared, which was almost absent in shock. We may mention that F. G. Akhmedov [1] observed strengthening of the depressor, and not the pressor, phase of the reflex under consideration. In this opinion, the cause of such a change in the pressor reflex is pessimal inhibition of the vasoconstrictor centers with simultaneous concomitant pessimal exaltation of the vasodilator centers. The author does not explain what is meant by the term "pessimal exaltation".

Investigation of the pressor reflexes caused by acute asphyxia showed that changes caused therein during lowering of the arterial pressure were similar to those in the pressor reflexes just examined. The results of one of these experiments are shown in Fig. 3.

The changes which we have described in the pressor vascular reflexes demonstrate that the capacity of the vasomotor center to respond to pressor stimuli is not merely restored when the arterial pressure is lowered, but is even increased by comparison with normal.

Attention is also directed to the fact that the rate of restoration of the magnitude of the pressor reflexes was considerably greater than the rate of restoration of the arterial pressure level. This means that the restoration of arterial pressure in anaphylactic shock is brought about, not by a passive increase in the mass of circulating blood, but by the active influence of the regulating mechanisms of the central nervous system. Under these circumstances, in view of the increased capacity of the vasomotor center to respond to pressor stimuli, favorable conditions are created for such restoration to take place.

It must also be mentioned that we were unable to observe any phasic parabiotic changes in the pressor vascular reflexes, whether in the initial period of lowering of the arterial pressure or during its restoration. Using stimulation of the vagus nerve of different intensities, in all cases we observed that the "rules of force" were obeyed.

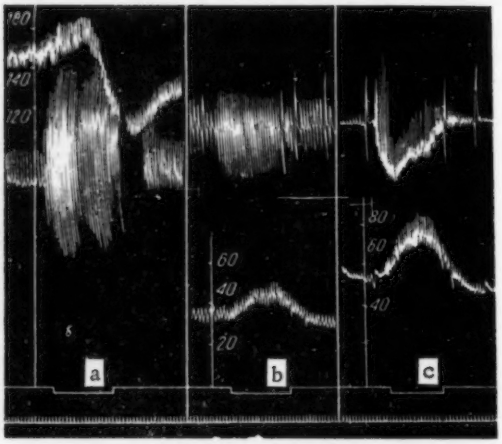


Fig. 2. Vascular reflex in response to stimulation of the central end of the sciatic nerve of a dog. a) In normal conditions; b) 20 minutes after injection of the assaulting dose of serum; c)30 minutes after. Legend as in Fig. 1, with the exception of a, in which respiration is traced below the arterial pressure.

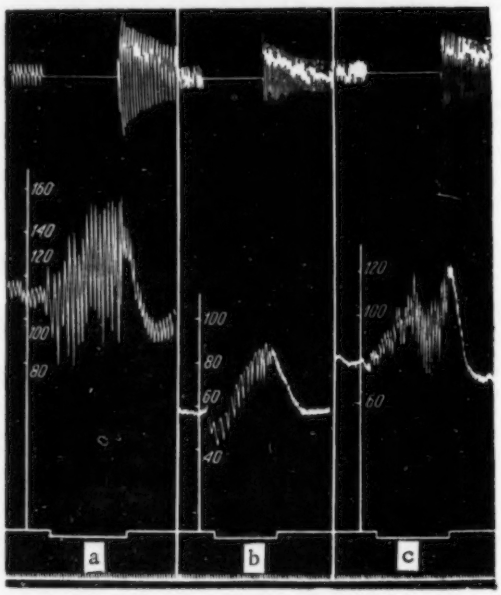


Fig. 3. Vascular reflex in response to acute asphyxia in a rabbit. a) In normal conditions; b) 15 minutes after injection of the assaulting dose of serum; c) 25 minutes after. Legend as in Fig. 1.

In consequence of this, it is difficult to agree with the opinion of V. S. Kiselev [7-10] and of other workers that, in anaphylactic shock, a parabiotic state develops in the central nervous system. Should it happen that when an animal is in a state of shock and parabiosis develops in the central nervous system, then, according to the teaching of N. E. Vvedenskii [2], any additional, intensive stimulation must of necessity deepen this state and lead to still further disturbance of the circulation. The results of our experiments, and certain reports in the literature, including those of V. S. Kiselev, do not, however, support this hypothesis. V. S. Kiselev [7], for instance, who regards shock as the development of a parabiotic state in the central nervous system, used the occipital injection of warm Ringer-Locke solution, stimulating the vasomotor and respiratory centers, to extricate animals from their state of shock.

The favorable influence of intraarterial fusion of blood or blood-substitutes, when used in the treatment of shock or hemorrhage [11], is due mainly to the reflex excitation of the vasomotor center in consequence of the intensive stimulation of the vascular receptors.

Finally, it must be stated that evidence has recently appeared [4, 13, 14] showing that an increase in the excitation of the central nervous system by some means or other may result in the extrication of an animal from a state of shock.

Reports in the literature and our own investigations thus show that in anaphylactic shock the vasomotor center does not lose its capacity to respond to pressor stimuli. This helps to explain the effectiveness of intraarterial infusion of blood or blood substitutes under pulsating pressure in conjunction with the use of stimulants in overcoming the low arterial pressure associated with moderately severe shock.

## SUMMARY

Experiments were conducted on rabbits and dogs under conditions of anaphylactic shock of medium intensity. The pressor reflexes inhibited at the beginning of the arterial hypotension not only recover their function but even become intensified, in comparison with the normal condition. This indicated that in the given state the ability of the vasomotor center to respond to the pressor stimuli is not only restored, but even intensified. Phasic parabiotic changes of the pressor reflexes were not observed either at the beginning of the reduced arterial pressure nor during its restoration. The stimulation of the corresponding efferent nerve by impulses of different intensity demonstrated that the so-called "rule of force" remained undisturbed.

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*BIOCHEMISTRY AND BIOPHYSICS*

# CHANGES IN THE BUFFER CAPACITY OF THE TISSUES DURING ENDOCRINE DISORDERS AND GROWTH OF TUMORS

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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In connection with data in the literature on changes in the acid-base balance in the tissues of malignant tumors [3-6], and also of data indicating the role of endocrine disorders in tumor development [1, 2, 7, 8], we considered that it would be of interest to investigate the changes in the buffer capacity of the tissues in association with such disturbances.

We could find no relevant information in the literature.

## METHOD

Experiments were conducted on white rats, mainly males weighing 250-300 g, and females weighing 200-250 g.

The total number of rats used in the investigation was 66, of which 12 were controls. Twelve underwent adrenalectomy and twelve thyroidectomy; nine were castrated, seven underwent Biskind's operation, nine had an M-1 sarcoma and six a sarcoma 45.

Endocrine disorders were induced by thyroidectomy, adrenalectomy, castration and also by castration followed by transplantation of the ovary to the spleen by Biskind's method.

Tumor formation was induced by means of the subcutaneous inoculation of a suspension of tumor tissue. For this purpose sarcomas of strains M-1 and 45 was used. The rats were sacrificed by decapitation: the adrenalectomized animals after 5-9 days, the thyroidectomized and castrated animals at the same time and also after 2-3 weeks, and those undergoing Biskind's operation were sacrificed 4-6 months after operation. The tumor-bearing animals were sacrificed 2-4 weeks after inoculation.

Weighed samples of tissue were taken from the brain, skeletal muscles, heart muscle, liver, kidneys, spleen, lungs and testicles of the sacrificed animals, and these were ground in a mortar to produce a homogeneous mass, to which was added physiological saline in the proportion of 10 ml solution to 1 g tissue. After standing for one

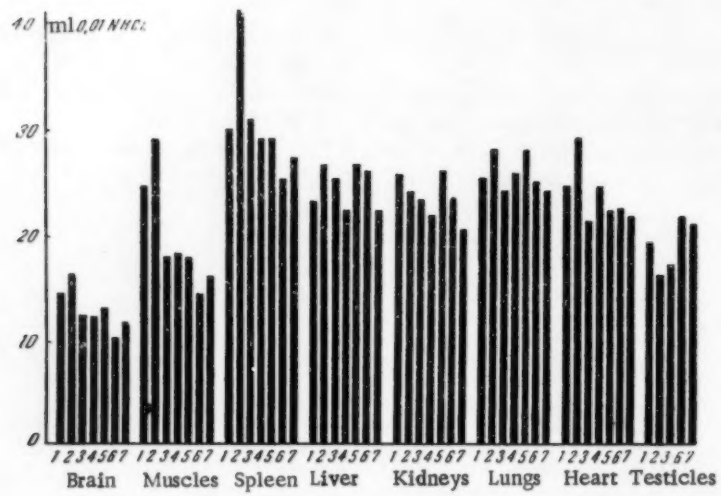


Fig. 1. Changes in the buffer capacity of the tissues (mean values). 1) Control animals; 2) adrenalectomized; 3) thyroidectomized; 4) castrated; 5) animals undergoing Biskind's operation; 6) animals with M-1 sarcoma; 7) animals with sarcoma 45.

hour the extract was separated by centrifugation for 20 minutes at 1500 rpm.

The buffer capacity of the tissue extracts thus obtained was investigated by the method of potentiometric titration with a glass electrode. The LP-5 lamp potentiometer was used. Titration was performed on 10 ml of extract, in which the volume of 0.01 N HCl necessary to produce a shift of one unit was determined. According to Van Slyke, this value is taken as a measure of the buffer capacity in relation to blood. Knowing the degree of dilution of the tissue extracts, the values of the buffer capacity that were found were converted for 100 ml of extract.

## RESULTS

Investigation of the tissue extracts of control rats showed that for each tissue there was a characteristic and definite value for the buffer capacity (Fig. 1). The smallest buffer capacity was found in the brain tissue and the highest in the spleen.

The buffer capacity of the tissues of the lungs and kidneys, i.e., of organs known to play an important part in the control of the reaction of the blood, was equal and on a relatively high level. The buffer capacity of the skeletal muscles was only slightly less than that of these organs.

This definite level of the buffer capacity of the tissues of other organs may possibly be connected with their functional properties. In this respect, the low buffer capacity of the brain tissues, which are known to respond to very small changes in the acid-base balance of the body, is of special interest. It must be pointed out here that an investigation of the buffer capacity of different divisions of the brain, carried out on a small number of animals, showed the smallest value of the buffer capacity to be in the brain stem.

Investigation of the buffer capacity of the tissues of adrenalectomized animals showed an increase in the buffer capacity of the tissues of the spleen, heart muscle, skeletal muscles, brain and liver. The buffer capacity of the kidney and lung tissues was only slightly changed, and that of the testicles was lowered (Figs. 1 and 2). In the thyroidectomized and castrated males, and also in the castrated females in which the ovary was transplanted into the spleen, a fall in the buffer capacity of the skeletal muscles, and then in the brain and kidney tissues was observed, while the buffer capacity of the remaining tissues was relatively constant (Figs. 1 and 2; 3, 4, and 5). The results obtained demonstrate that endocrine disturbances caused characteristic changes in the buffer capacity of the various tissues.

During the investigation of the buffer capacity of the tissues of the tumor-bearing animals, its value was found to be considerably decreased, especially in the skeletal muscles, and then in the tissues of the brain and also of the spleen and liver (Figs. 1 and 2; 6 and 7). It may be postulated that the sharp fall observed in the buffer capacity of the skeletal muscles was due to the fact that, in the process of its growth, the tumor developing in the body used up the vast reserves of buffer materials in the skeletal muscles, forming about one-half of the mass of the body.

It will be seen from the results described that the changes in the buffer capacity of the tissues of the tumor-bearing animals were similar to those observed after castration and thyroidectomy, but were expressed to a greater degree.

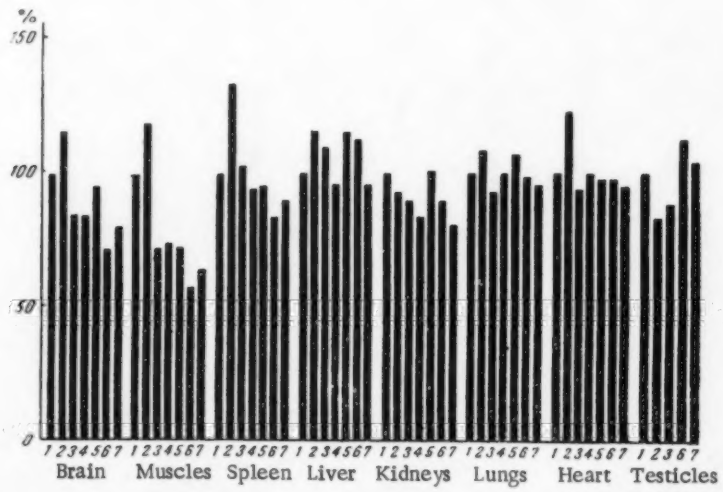


Fig. 2. Changes in the buffer capacity of the tissues of experimental rats as a percentage of the change in the buffer capacity of the tissues of the control rats (mean values). Legend as in Fig. 1.

So far as the active reaction (pH) of the tissues is concerned, this remained constant for the same tissues, both in intact animals, in animals with endocrine disturbances and in tumor-bearing animals.

## SUMMARY

The authors studied the buffer capacity in the tissues of animals with experimentally induced endocrine disturbances and experiments M-1 and sarcoma 45 by the method of potentiometric titration with the aid of a glass electrode. An increase of the buffer capacity in all the tissues was observed following adrenalectomy. Similar changes were noted in thyroidectomy, castration and tumor growth, i.e., reduction of the buffer capacity, mainly in the skeletal muscle tissue as well as in tissues of the brain, spleen, liver and kidneys. The decrease was even more marked in animals with tumors.

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# THE EFFECT OF CERTAIN PHENOTHIAZINE DERIVATIVES ON RESPIRATORY PHOSPHORYLATION IN THE CARDIAC MUSCLE OF THE RABBIT

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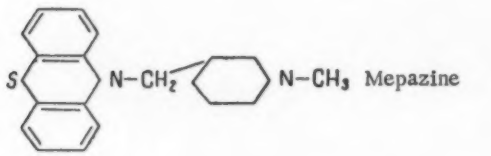
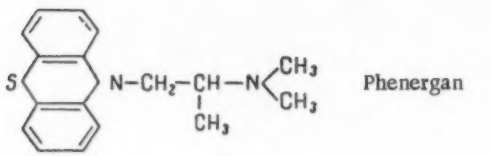
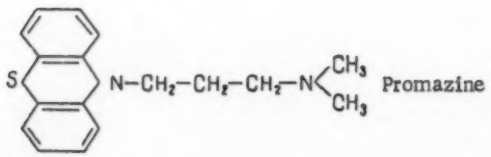
Certain derivatives of the phenothiazine series, possessing a neuroplegic action, are widely employed in clinical practice. In this connection the attention of biochemists has been drawn to the study of the effect of phenothiazine derivatives on a number of enzymic processes. The drug most studied in this respect is aminazin (chlorpromazine). Data are available showing the inhibition by chloropromazine of respiration [7], phoshporylation [3, 5, 9], adenosinetriphosphatase [3, 8, 9] and cytochrome oxidase [7, 6] activity, and phospholipid metabolism [4]. Regarding the analogs of chloropromazine – promethazine (phenergan) and promazine, those are known to depress cytochrome oxidase and adenosinetriphosphatase [8].



Mepazine



Phenergan



Promazine

In the present research we investigated the effect of three phenothiazine derivatives – mepazine [(acetate-10-) N-methylpiperidyl-3-methylphenothiazine], phenergan [(hydrochloride-10-) 2-dimethylaminopropylphenothiazine] and promazine [(hydrochloride-10-) 3-dimethylaminopropylphenothiazine] – on the processes of respiration and the accompanying phosphorylation in homogenates of the cardiac muscle of the rabbit.

## METHOD

Male rabbits weighing 2.0-2.5 kg were decapitated, the heart was rapidly extracted and placed in cold physiological saline and, through a cannula introduced into the aorta, it was perfused in order to free it from the greater part of its blood. The cardiac muscle was then separated from connective tissue and fat, cut into small pieces with scissors and homogenized for one minute in the cold in a glass homogenizor in two volumes (in proportion to the weight of tissue) of a saline medium. The homogenate used in the experiments was filtered through several layers of gauze. The concentrations of the components of the saline medium for homogenization were as follows: NaCl – $1.4 \cdot 10^{-1}$ M, $MgSO_4$ – $2 \cdot 10^{-3}$ M, KCl – $5 \cdot 10^{-3}$ M. The reaction of the medium was adjusted to pH = 7.8-8.0 with caustic soda (0.5 N). The experiments on oxidative phosphorylation were conducted in Warburg flasks. Each flask contained 1 ml of incubation medium, consisting of 0.5 ml of homogenate and 0.5 ml of buffer solution (pH = 8.0). The concentrations of the various components of the buffer solution were: $Na_2HPO_4$ – $4.6 \cdot 10^{-2}$ M, $MgSO_4$ – $2 \cdot 10^{-3}$ M, KCl – $5 \cdot 10^{-3}$ M, NaCl – $1 \cdot 10^{-1}$ M. Creatine, which was used as the final phosphate acceptor, was added in an amount of 5 mg of a sample volume of 1 ml. The phenothiazine derivatives were introduced into the composition of the buffer solution.

The experiments were performed in an atmosphere of oxygen, the absorption of which was measured in the Warburg apparatus. Incubation was at 260° for 18 minutes. The intensity of phosphorylation was judged by the formation of phosphocreatine and by the decrease in inorganic phosphorus in the medium after incubation. At the end of incubation the proteins were precipitated by an equal volume of 5% trichloroacetic acid, and separated by centrifugation. The phosphocreatine of the supernatant fluid was determined by A. M. Alekseeva's method [1] and the inorganic phosphate by the Fiske-Subbarow method [2]. The experiments on the study of the oreatine kinase activity were carried out on muscle extracts [2]. Minced

muscle from the washed heart was extracted in the cold with two volumes of distilled water for 20 minutes. The tissue was then squeezed between gauze, the extract centrifuged and the supernatant fluid was used in the experiments as an enzyme solution. The experimental samples consisted of 0.5 ml of extract and 0.5 ml of buffer solution, to which had previously been added adenosinetriphosphate (10 mg), creatine (5 mg) and NaF (1 mg). In the experiments on the study of the adenosinetriphosphatase activity, a homogenate of cardiac muscle was used as enzyme solution. The experimental samples consisted of 0.5 ml of homogenate and 0.5 ml of buffer solution, to which had been previously added adenosinetriphosphate (10 mg) and monoiodoacetate (0.2 mg).

The phenothiazine derivatives were introduced into the composition of the buffer solution, consisting in this case of $1.5 \cdot 10^{-1}$ M $NaHCO_3$ solution in a 1% solution of $MgSO_4$. After the additives had been dissolved the reaction of the medium was adjusted to pH = 8.0.

Incubation was at 26° for 18 minutes. At the end of incubation, the phosphocreatine content of protein-free centrifugates was determined (in the experiments to study creatine kinase activity) and the increase in mineral phosphorus (in the experiments to study adenosinetriphosphatase activity).

## RESULTS

The effect of all three phenothiazine derivatives on respiration and phosphorylation is shown in Fig. 1.

phosphorylation (at a concentration of $0.8 \cdot 10^{-3}$ M) and of the less sharp lowering of phosphorylation than of respiration (at a concentration of $1.2 \cdot 10^{-3}$ M), the P : O coefficient was increased with low concentrations of promazine.

Mepazine, in a concentration of $0.8 \cdot 10^{-3}$ M, slightly depressed respiration and stimulated phosphocreatine formation. Concentrations exceeding $0.8 \cdot 10^{-3}$ M caused considerable depression of phosphocreatine formation. Particularly marked depression of phosphocreatine formation was caused by mepazine in a concentration of $1.6 \cdot 10^{-3}$ M; at this concentration dissociation of respiration and phosphorylation took place, expressed as a fall in the P : O coefficient.

In contrast to promazine and mepazine, phenergan in a concentration of $0.8 \cdot 10^{-3}$ M depressed respiration and phosphocreatine formation equally. In concentrations higher than $0.8 \cdot 10^{-3}$ M, and especially above $1.2 \cdot 10^{-3}$ M, phenergan depressed phosphorylation more strongly than respiration, as a result of which the P : O coefficient fell considerably.

In order to discover whether the depression of phosphocreatine formation was the result of inhibition of oxidative phosphorylation or inhibition of creatine kinase, or whether it arose as the result of activation of adenosinetriphosphatase, we carried out experiments in which we studied the effect of the phenothiazine derivatives on the activity of creatine kinase and adenosinetriphosphatase.

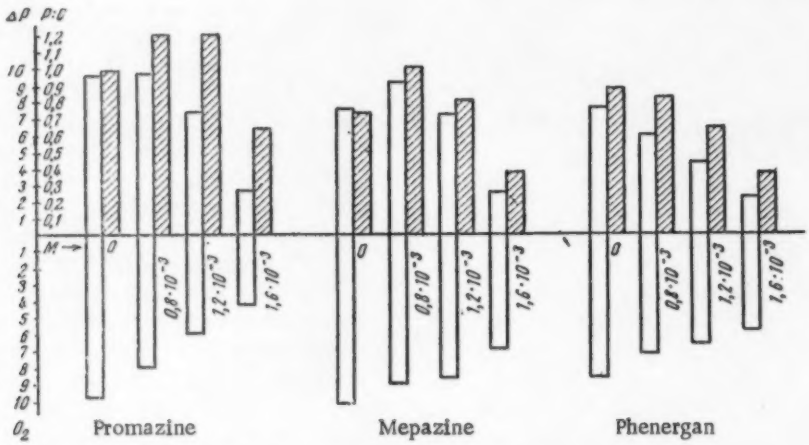



Fig. 1. The effect of different concentrations of phenothiazine derivatives on oxidative phosphorylation. ΔP – Formation of phosphocreatine in microatoms of phosphorus; $\Delta O_2$ – absorption of oxygen in microatoms; shaded columns – P : O coefficient.

It can be seen that promazine, in a concentration of $0.8 \cdot 10^{-3}$ M, depressed respiration while the phosphocreatine formation was increased or remained within normal limits. Inhibition of phosphocreatine formation by promazine began when its concentration was increased further (above $0.8 \cdot 10^{-3}$ M). As a result of the more pronounced lowering of respiration and the slight increase in

The results of these experiments, shown in Figs. 2 and 3, demonstrate that mepazine, phenergan and promazine (in concentrations depressing oxidative phosphorylation) neither depressed creatine kinase nor activated adenosinetriphosphatase. It was characteristic that, in the concentrations used, mepazine and promazine showed a depressing action on adenosinetriphosphatase activity.

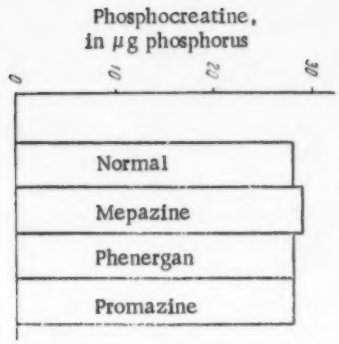



Fig. 2. Effect of mepazine, phenergan and promazine (in concentrations of $1.6 \cdot 10^{-3}$ M) on creatine kinase activity.

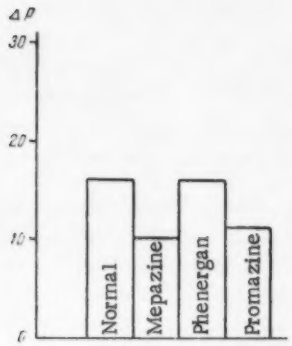



Fig. 3. Effect of mepazine, phenergan and promazine(in concentration of $1.6 \cdot 10^{-3}$ M on adenosinetriphosphatase activity. P – increase in mineral) phosphate (in microatoms).

It may be concluded from the results obtained that the mechanism of action of the tested phenothiazine derivatives on respiratory phosphorylation differed slightly. In the presence of mepazine, respiration was depressed to a lesser degree than in the presence of the two isomers investigated – phenergan and promazine, and promazine had a more pronounced effect than phenergan.

The action of mepazine was also characterized by stimulation of phosphorylation at low concentrations of the drug, with subsequent depression of the process as the concentrations were increased. Phenergan depressed phosphorylation in low concentrations, whereas promazine had no effect on this process in these concentrations. A special feature of promazine was the sharp depression of respiration, preceding the depression of phosphorylation.

By their effect on phosphorylation, the drugs tested could be placed in order: Mepazine in a concentration of $0.8 \cdot 10^{-3}$ M increased phosphorylation; promazine increased it only slightly, and phenergan depressed it. In concentration of over $0.8 \cdot 10^{-3}$, phenergan depressed phosphorylation to a greater degree than promazine and mepazine.

The difference between the effect of the tested phenothiazine derivatives on respiration and phosphorylation is accounted for by differences in the chemical structure of these substances. It may be postulated that the presence of an N-methylpiperidyl-3-methyl side-chain in the structure of mepazine explains the dissociating effect of this preparation on respiration and the associated phosphorylation. On replacement of the mepazine side-chain by a 2-dimethylaminopropyl or a 3-dimethylaminopropyl chain, the capacity of the preparations to dissociate respiration and phosphorylation is reduced.

## SUMMARY

Phenothiazine derivatives, mepazine and promazine, when added to homogenates of cardiac muscle of the rabbit in a concentration of $0.8 \cdot 10^{-3}$ M, cause a rise in the efficacy of oxidative phosphorylation, increasing the ΔP and the P : O coefficient. In a $1.2 \cdot 10^{-3}$ M concentration of mepazine and promazine ΔP is reduced, though to a lesser degree than $\Delta O_2$, the P : O coefficient remaining slightly above the normal level.

A $0.8 \cdot 10^{-3}$ M phenergan concentration uniformly reduces both respiration and phosphorylation. With a further rise of the phenergan concentration phosphorylation decreases more sharply than respiration. High concentrations of mepazine, phenergan and promazine ($1.6 \cdot 10^{-3}$ M) sharply depress phosphocreatine formation and cause a decrease of P : O. The phenothiazine derivatives neither depress creatine kinase nor activate adenosinetriphosphatase.

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# THE EFFECT OF COMPOUNDS DEPRESSING MITOTIC CELL DIVISION ON THE RESPIRATION OF RAT LIVER SLICES

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In previous research by one of the authors (Hadnad [1]) it was observed that certain drugs, acting as antimitotic poisons, had an inhibiting action on phosphorylation. It therefore appeared to be of interest to investigate the action of these antineoplastic substances on tissue respiration.

## METHOD

Experiments were carried out on rats which were sacrificed after starvation for 24 hours. The liver was extracted and cut into slices weighing 0.2 g, and the $O_2$ absorption and $CO_2$ excretion by these slices were estimated in a Warburg apparatus in a buffered solution (pH 7.38). The effect of the following antitumor drugs was investigated: nitrogen mustard (Boots, Merck), urethane, colchicine, sarcomycin and degranol. Degranol is a nitrogen-mustard derivative (1, 6-desozy-D-mannitol-dihydrochloride), synthesized by Varga and tested at the Budapest Oncological Institute [2, 3]. It has been found to have low toxicity and has given good clinical results. Sarcomysin is an antitumor antibiotic, isolated by Hamao Umezawa from a strain of Streptomyces erythrochromogenes. Degranol was added to the Warburg flask in a dose of 10-200 γ, nitrogen mustard - 50-400 γ, urethane - 1-50 mg, colchicine - 0.1-10 γ and sarcomycin - 25-400 γ. Two to five parallel investigations were made with each concentration of the respective drug; mean results are given in the table.

TABLE. Effect of Antimitotic Drugs on the Respiratory Coefficient of Rat Liver Slices

| Degranol | | | Nitr. Must. | | | Urethane | | | Colchicine | | | Sarcomycin | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| quant. of drug added to Warburg flask (γ) | respiratory coefficient | number of tests | quant. of drug added to Warburg flask (γ) | respiratory coefficient | number of tests | quant. of drug added to Warburg flask (γ) | respiratory coefficient | number of tests | quant. of drug added to Warburg flask (γ) | respiratory coefficient | number of tests | quant. of drug added to Warburg flask (γ) | respiratory coefficient | number of tests |
| 10 | 0,98 | 4 | 50 | 0,90 | 5 | 10 | 0,65 | 5 | 0,001 | 0,88 | 5 | 50 | 1,01 | 7 |
| 25 | 0,97 | 3 | 100 | 0,40 | 9 | 20 | 0,69 | 7 | 0,01 | 0,46 | 9 | 100 | 0,44 | 13 |
| 50 | 0,39 | 10 | 200 | 0,53 | 6 | 50 | 0,76 | 8 | 0,05 | 0,38 | 7 | 200 | 0,54 | 8 |
| 100 | 0,37 | 8 | 400 | 0,33 | 5 | | | | 0,1 | 0,30 | 15 | 400 | 0,10 | 10 |
| 200 | 0,40 | 7 | | | | | | | 1,0 | 0,47 | 13 | | | |
| | | | | | | | | | 2,5 | 0,48 | 19 | | | |
| | | | | | | | | | 5 | 0,41 | 13 | | | |
| | | | | | | | | | 10 | 0,48 | 11 | | | |

## RESULTS

Results relating to the effect of the substances which we tested on the respiration of the liver slices are shown in the table. It will be seen from this table that, in certain concentrations, these substances sharply lowered the respiratory coefficient.

We cannot yet give a precise explanation of the phenomenon observed, but we consider that the changes in this coefficient are the result of disturbance of the carbohydrate metabolism of the tissues.

The better therapeutic results from degranol than from nitrogen mustard are probably due to its low toxicity so that it could be given in doses ten times larger than nitrogen mustard. The effect of degranol of tissue metabolism, however, is far less pronounced than that of nitrogen mustard.

## SUMMARY

The authors studied the effect of substances depressing the mitotic division of the cells, and employed as anticancer preparations, on the formation of $CO_2$ and the oxygen intake by slices of rat liver. The addition of degranol (50-200 γ) nitrogen mustard (100-400 γ), urethane (10-50 mg), colchicine (0.01-10 γ) and sarcomycin (100-400 γ) to 0.2 gm of the liver had no effect on its oxygen intake. However, the oxygen production by the liver was greatly reduced, resulting in a marked drop of the tissue respiratory coefficient, which then assumed very low values. With the addition of degranol this coefficient fell down to 0.037, with that of nitrogen mustard - to 0.33 urethane - to 0.65 colchicine - to 0.30 and of sarcomycin - to 0.10.

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*PHARMACOLOGY*

# A COMPARISON OF THE GANGLIOBLOCKING AND CURARIFORM EFFECTS OF 3,4-DITHIAHEXANE-1,6-BIS-TRIMETHYLAMMONIUM DIIODIDE AND HEXONIUM [HEXAMETHONIUM]

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Bis-trimethylammonium derivatives in which the trimethylammonium groups are linked by a polymethylene chain have recently been thoroughly investigated in regard to their ganglioblocking and curariform effects [see 4]. Dithio analogs of these compounds of the general type:

$$(CH_3)_3\overset{+}{N} - (CH_2)n - S - S - (CH_2)n - \overset{+}{N}(CH_3)_3 \cdot 2\bar{J}.$$

have not been as thoroughly studied.

Hunter [see 3] examined 5,6-dithiadecane-1,10-bis-trimethylammonium and found that it is close to decamethonium, both quantitatively and qualitatively. There are brief data [see 2] concerning the ganglioblocking activity of 3,4-dithiahexane-1,6-bis-trimethylammonium which indicate that this compound is less active than hexamethonium. 3,4-Diethiahexane-1,6-bis-trimethylammonium has not been investigated in any greater detail.

In this work, the purpose of which was to compare the pharmacological activity of hexamethonium (Hexonium) with that of its dithio anolog, we studied the comparative effect of these compounds on ganglionic and neuromuscular synapses. We made an analogous study of 4,5-dithiaoctane-1,8-bis-trimethylammonium diiodide.

Hexonium (diiodide) was obtained from S. V. Anichkov's (Active Member of the AMN SSSR) laboratory. 3,4-Dithiahexane-1,6-bis-trimethylammonium diiodide, hereinafter referred to as Dithiahexonium, and 4,5-dithiaoctane-1,8-bis-trimethylammonium diiodide were synthesized by the chemist R.G. Kostyanovskii. The dithio derivatives are colorless needles, readily soluble in water. The melting point of Dithiahexonium is 233°, that of the dithiaoctane derivative, 203-205°.

## METHOD

Cats anesthetized with urethan (1 g/kg intraperitoneally) were used to study the effect of the preparations on the conduction of stimulation in the ganglia and neuromuscular synapses.

The change in the reaction of the superior cervical ganglion indicated the effect on the sympathetic ganglia. Stimulation of the preganglionic trunk of the sympathetic nerve (with electric impulses) produced a prolonged tonic contraction of the nictitating membrane, which was recorded on a kymograph tape. Solutions of the preparations were introduced into the femoral vein. Inhibition of conductivity in the ganglia induced a decrease in the tonus of the membrane or the complete relaxation of the latter. We also studied the effect of the preparations on the changes in contractions of the nictitating membrane induced by intravenous injections of cytisine (20 μ g/kg). The arterial pressure and the respiration were recorded synchronously with the contractions of the nictitating membrane.

The effect on the parasympathetic ganglia (cardiac ganglia of the vagus nerve) was measured by the changes in the depressor reaction induced by stimulation of the peripheral section of the cervical trunk of the vagus nerve.

The changes in neuromuscular conductivity were judged according to the decrease in the amplitude of the gastrocnemius muscle's contractions in response to stimulation of the peripheral section of the sciatic nerve with electric, square-wave pulses given every 5 seconds and lasting 10 microseconds each. We also studied the effect of the preparations on a frog's isolated rectus abdominis muscle.

White mice intraveneously injected with the preparations were used to study the toxicity of the latter.

## RESULTS

Effect on the sympathetic ganglia. After the administration of Dithiahexonium in a dose of 0.1 mg/kg, the nictitating membrane relaxed slightly; in this dose, the preparation had an effect lasting 5-7 minutes. The effect of the dithiaoctane analog in the same dose was similar in intensity and duration, but Hexonium produced the same effect when used in a dose of 0.03 mg/kg (Fig. 1).

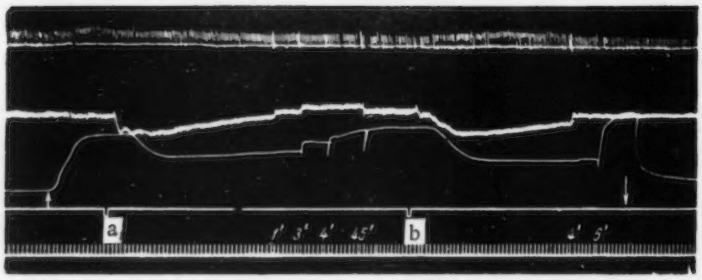


Fig. 1. Effect of Dithiahexonium and Hexonium on conduction of nervous excitation in the superior cervical ganglion. Experiment on cat weighing 3.4 kg. Urethan anesthesia. Curves show (from top to bottom): Respiration, arterial pressure; tonus of nictitating membrane; Dithiahexonium injection (a) – "OF 1465", 0.1 mg/kg and Hexonium injection (b) – 0.03 mg/kg; indications of time in 5-second marks and stopping of kymograph. The arrows (↑ ↓) mark the beginning and end of stimulation of the sympathetic nerve.

Dithiahexonium had a stronger effect lasting 10-12 minutes when its dose was increased to 0.2 and 0.3 mg/kg. Effects of similar force and duration were produced by Hexonium in doses of 0.075 and 0.1 mg/kg respectively.

Therefore, although Dithiahexonium has a pronounced ganglioblocking effect, it is approximately 1/3 as active as Hexonium. The effect of the dithiaoctane analog is the same as that of Dithiahexonium.

In a dose of 0.2 mg/kg, Dithiahexonium and the dithiaoctane derivative lessened the contraction of the nictitating membrane, the pressor reaction and the respiratory stimulation induced by the intravenous injection of cytisine. In this dose, the two preparations were only effective during the first few minutes after their administration; cytisine's effect was fully restored after 10-12 minutes.

Hexonium had an effect similar in intensity and duration when used in a dose of 0.05 mg/kg. When the dose of Hexonium was raised to 0.2 mg/kg, the preparation caused a prolonged (lasting more than an hour) reduction of the reactions of the arterial pressure, respiration and nictitating membrane to the injection of cytisine.

Effect on the parasympathetic ganglia. In a dose of 0.1 mg/kg, Dithiahexonium induced a 15-20% decrease in the depressor reaction of the arterial pressure in response to electric stimulation of the vagus nerve. The reaction was restored after 5-6 minutes. Hexonium had an analogous effect in a dose of 0.03 mg/kg.

In a dose of 0.3 mg/kg, Dithiahexonium caused a 30-33% decrease in the depressor reaction; the original reaction was restored 10-12 minutes after the injection of the preparation. The dithiaoctane analog had the same effect.

In a dose of 0.1 mg/kg Hexonium caused the depressor reaction to decrease more than 50%, and 50-60 minutes later, the original value of the reaction had not yet been restored.

Therefore, the effect of Dithiahexonium and its dithiaoctane analog on the parasympathetic ganglia is less than 1/3 as strong as that of Hexonium.

Effect on neuromuscular conductivity. In doses of 0.4-0.8 mg/kg, Dithiahexonium decreased the amplitude of muscle contractions and, in some experiments, effected a complete block of neuromuscular conductivity (Fig. 2,a). The effect of the preparation in these doses lasted 12-20 minutes. In doses of 1 mg/kg or more, Dithiahexonium not only blocked neuromuscular conductivity, but usually caused a 5-8 minute respiratory arrest.

Hexonium had considerably less effect on neuromuscular conductivity. Only after the injection of Hexonium in a dose of 20 mg/kg did we observe a brief (lasting 15-20 seconds) decrease in the amplitude of the muscle contractions. A greater decrease in the amplitude of the contractions was observed after the injection of Hexonium in a dose of 40 mg/kg (Fig. 2,b).

Therefore, the effect of Dithiahexonium on neuromuscular conductivity is more than 100 times stronger than that of Hexonium. The dithiaoctane analog was found to be even more active. In a dose of 0.1-0.2 mg/kg, it produced the same effect as Dithiahexonium in a dose of 0.4-0.8 mg/kg (Fig. 2,c).

Because of the strong inhibitory effect of Dithiahexonium on neuromuscular conductivity, it was interesting to determine the character of the curariform effect exerted by this compound. Curariform preparations of the bis-trimethylammonium series (decamethonium) are known

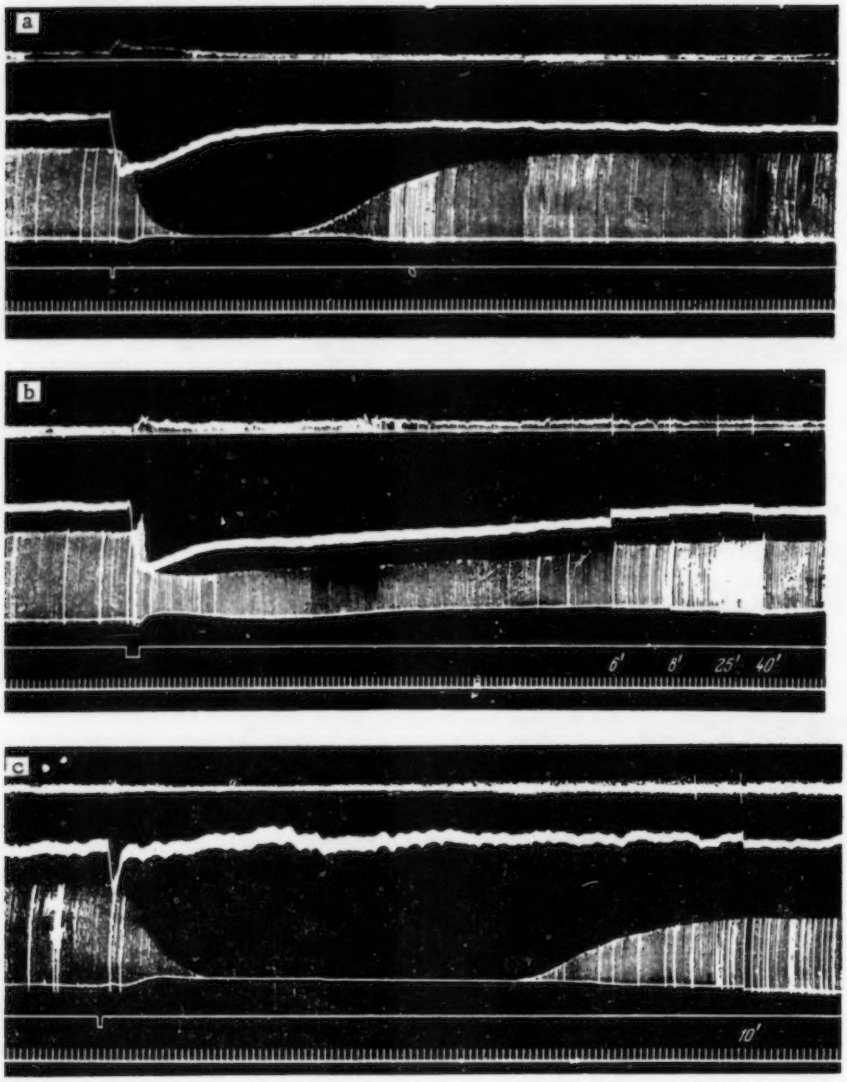


Fig. 2. Effect of the experimental preparations on neuromuscular conductivity. Experiments on a cat weighing 2.6 kg. Urethan anesthesia. Curves (from top to bottom) show: respiration, arterial pressure, contraction of gastrocnemius muscle, administration of substances, time in 15-second marks. a) Effect of Dithiahexonium ("OF 1465" – 0.4 mg/kg); b) – effect of Hexonium (40 mg/kg); c) effect of dithiaoctane analog ("OF 1521" – 0.12 mg/kg).

to belong to the group of substances having the depolarizing type of action. In experiments performed on cats, we found that the blocking effects of Dithiahexonium and the dithiaoctane analog are intensified by the intravenous injection of Proserine [neostigmine] (0.15 mg/kg). The administration of Diplacin [curare simulant] under the same experimental conditions (dose = 0.2-0.8 mg/kg) weakened the blocking effects of Dithiahexonium and the dithiaoctane analog.

Therefore, the effect of these two dithia derivatives is like that of the depolarizing substances.

It should be mentioned that the dithiaoctane derivative, when used in a concentration of at least $1 \cdot 10^{-5}$, caused a frog's rectus abdominis muscle to contract. Dithiahexonium (in concentrations of $1 \cdot 10^{-6}$ to $2 \cdot 10^{-3}$) caused no apparent contraction of the muscle.

Toxicity for white mice. Experiments were performed on mice weighing 16-17 g. Each dose was tested on 5 animals, which were observed for a period of three days from the time the preparations were administered.

The first signs of intoxication appeared after the injection of Dithiahexonium in a dose of 7.5 mg/kg. We observed inhibition, mild tremor and unsteady gait in the mice; with larger doses (10-12.5 mg/kg), brief respiratory arrest occurred. The $LD_{50}$, calculated according to Kerber's method, equalled 14.8 mg/kg. The $LD_{50}$ of the dithiaoctane derivative was 4.7 mg/kg.

Hexonium caused inhibition, tremor and, in large doses (60-70 mg/kg), unsteady gait in the mice. The absolute lethal dose was 80 mg/kg. The animals died with symptoms of respiratory arrest 2-7 minutes after the injection. The $LD_{50}$, calculated according to Kerber's method, equalled 62.8 mg/kg.

Therefore, Dithiahexonium was found to be more than 4 times more toxic to white mice than Hexonium, while the dithiaoctane analog was more than 13 times more toxic than the latter.

The investigations conducted show that the substitution of the two $CH_2$ groups in the polymethylene chain of Hexonium by two sulfur atoms substantially alters the pharmacological activity of the compound, changing both the ganglioblocking and the curariform activity of the preparation.

The ganglionic properties are decreased. Our experiments with the tonicized membrane and the injection of cytisine showed that Dithiahexonium's effect on the sympathetic ganglia is at least 1/3 as strong as that of Hexonium; the experiments studying the effect on the parasympathetic ganglia of the cardiac branches of the vagus nerve gave approximately analogous results.

Curariform activity, on the other hand, increases considerably. Our experiments showed that the effect of Dithiahexonium on neuromuscular conductivity is more than 100 times stronger than that of Hexonium.

According to the data of Paton and Zaimis [4] and of Barlow and Ing [1], the most active ganglioblocking substances of the bis-trimethylammonium derivatives are compounds with five or six methyl groups, in which the distance between the quaternary nitrogen atoms is about 7.7 A. Compounds in which the distance between the nitrogen atoms is about 14-15 A possess a pronounced curariform activity. In Dithiahexonium, this distance is 8.76 A. Therefore, the distance between the quaternary nitrogen atoms is essentially the same in both Dithiahexonium and Hexonium. These two compounds differ substantially in pharmacological activity: Dithiahexonium's ganglioblocking activity is weaker than that of Hexonium, but its effect on the neuromuscular synapses is considerably stronger than that of the latter.

Moreover, Dithiahexonium is considerably more toxic than Hexonium.

Although the dithiaoctane derivative has about the same effect as Dithiahexonium on ganglia, its curariform activity and toxicity are considerably greater.

## SUMMARY

The authors studied the effect of the following preparations on the sympathetic and parasympathetic ganglia and on neuromuscular transmission: Hexonium, 3,4-dithiahexane-1,6-bis-trimethylammonium diiodide (Dithiahexonium) and 4,5-dithiaoctane-1,8-dis-trimethylammonium diiodide.

The effect of Hexonium on the gangli is about triple that of Dithiahexonium. The latter is more active than Hexonium in its effect on neuromuscular transmission (approximately 100 times). Dithiahexonium is 4 times more toxic than Hexonium.

The dithiaoctane derivative does not significantly differ from Dithiahexonium as to effect on ganglia, but possesses a higher curare-like effect and toxicity.

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# THE EFFECT OF 2-METHYLNAPHTHOQUINONE ON THE PARASYMPATHETIC INNERVATION AND CARDIAC ACTIVITY OF COLD-BLOODED ANIMALS

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2-Methyl-1,4-naphthoquinone (Methinone [Menadione]) is a synthetic preparation of vitamin K, the role of which in the mechanism of blood coagulation is well known. 2-Methylnaphthoquinone has also been found to be a peculiar inhibitor of the acetylcholine metabolism, inhibiting the synthesis of acetylcholine by blocking the enzyme choline-acetylase [17, 18, 20]. Because of this property, Methinone can be used to demonstrate the role of acetylcholine in elements of the somatic nervous system [3, 19].

Investigation of Methinone's effect on the heart, the classic object of parasympathetic innervation, is of great interest in this connection, especially since the question of the mechanism of the effect exerted by parasympathetic nerves on the heart and the role of acetylcholine in this mechanism is still under discussion [5, 6, 10, 11, 12].

## METHOD

Experiments were performed on a frog's heart (R. ridibunda). In some cases, we used a heart isolated according to Shtraub, with intact innervation; in other cases, the heart action was studied under conditions of natural circulation. In the latter experiments, the heart and the vagosympathetic trunks were dissected out without disturbing the central nervous system. The nerves were stimulated with Kronecker's inducing apparatus, powered by a 4-volt storage battery.

We studied the effect on the isolated heart of 2-methylnaphthoquinone solutions in concentrations of 1 : 10,000-1 : 100,000; the original Methinone solution was prepared in a boiling Ringer's solution in a dilution of 1 : 5000. For intravenous administration, we used a saturated solution of 2-methylnaphthoquinone in a dose of 3 ml, i.e., 0.1 mg of the substance per 10 g of frog weight. More than 100 experiments were performed.

## RESULTS

Experiments on isolated heart. The way in which the cardiac activity and the effect of the extracardial nerves changes under the influence of 2-methylnaphthoquinone depended on the concentration of the solution used. Low concentrations (1 : 50,000- 1 : 100,000) usually did not cause changes in the frequency or force of the cardiac contractions (Fig. 1,a,b). After Methinone was introduced into the cannula, however, we observed marked changes in the parasympathetic effects upon stimulation of the vagosympathetic trunk. These changes primarily affected the inotropic component of the parasympathetic effect; it decreased quickly, then disappeared completely during the first 5-10 minutes. In this case, the amplitude of the contractions did not decrease as usual during stimulation of the nerves, but remained unchanged (Fig. 1,b). The chronotropic component of the vagal effect was more stably retained, being maintained in many cases throughout the experiment, although considerably reduced in degree.

The vagal arrest of the heart proved to be of medium stability. Over a period of 30-50 minutes, its duration gradually decreased (Fig. 1,b) until, about the end of the first hour, it usually disappeared completely. At this time, stimulation of the vagosympathetic trunk either did not cause parasympathetic effects or caused only a slight retardation of the rate of the contractions. Under these conditions, stronger stimulation did not change the character of the heart's reaction. This and the fact that the force of the threshold stimulation did not change during the experiment indicate that the disturbances observed with the action of Methinone on the isolated heart were not connected with changes in the excitability of the parasympathetic nerves.

These same concentrations of Methinone did not cause any essential changes in the sympathetic effect, which remained after the vagal effect had completely disappeared (Fig. 1, c).

When Methinone was used in a concentration of 1 : 50,000, all the changes described developed more rapidly and were more apparent than with the 1 : 100,000 concentration. Moreover, in some experiments in which the 1 : 100,000 Methinone solution was used, we observed the spontaneous restoration of the vagal effect after its disappearance, which never happened when the 1 : 50,000 concentration was used.

Washing out with a Ringer's solution restored all the parasympathetic effects except the negative inotropic effect (Fig. 1, d).

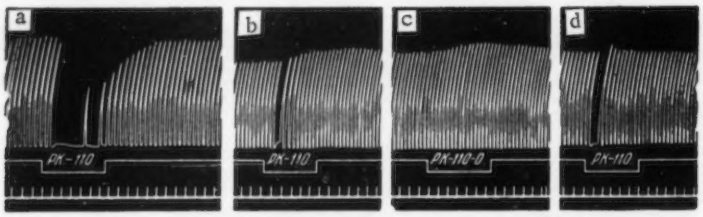


Fig. 1. Effect of Methinone (1 : 50,000) on the parasympathetic effects of an isolated heart. a) Before action of Methinone; b) 25 minutes after introduction of Methinone solution into cannula; c) after 45 minutes; d) 15 minutes after start of washing out the heart with a Ringer's solution. Curve (from top to bottom) show: recording of heart contractions, indication of stimulation of vagosympathetic trunk, time indicated in 5-second marks.

The changes caused by the higher concentrations of Methinone (1 : 10,000-1 : 20,000) were primarily the same in type and sequence as those caused by the lower concentrations, but were quicker to develop. The inotropic component of the vagal effect disappeared almost immediately after the introduction of Methinone, during the first few minutes of its action. The chronotropic component and cardiac arrest were observed for a somewhat longer period, up to 10-20 minutes. In contrast to the preceding experiments, the prolonged action of 2-methylnaphthoquinone in the higher concentrations caused irreversible depression of the parasympathetic effects. Another peculiarity of the effect of these concentrations was the fact that after the disappearance of the vagal effects, the sympathetic effects also became depressed.

The disturbances which occurred in the extracardial innervation with the action of the higher Methinone concentrations took place on a background of acute change in the heart's contractility and automatism. The amplitude of the contractions decreased, and their rhythm became progressively slower (Fig. 2, a,b). Subsequently, acute disturbances in the conduction of automatic impulses developed, causing disaaciation of the auricular and ventricular contractions,and, later, the development of atrioventricular block (Fig. 2,c,d,e). Ventricular arrest usually occurred during this period, and while the less frequent sinal automatism was preserved; in some cases, the automatism of the sinus and ventricle each had an independent rhythm.

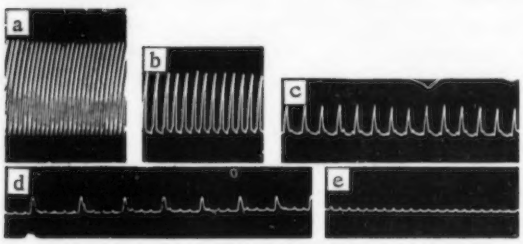


Fig. 2. Change in the activity of an isolated heart caused by high concentrations (1 : 10,000) of Methinone. a) Before introduction of Methinone; b,c,d) 40, 60, and 80 minutes respectively, after introduction of Methinone solution into cannula; e) the same after 100 minutes (auricles only contracting). Cylinder rotation rate the same as in Fig. 1.

Experiments introducing 2-methylnaphthoquinone into the blood. The introduction of Methinone into the blood also disturbed the parasympathetic effects. The vagal cardiac arrest usually disappeared five minutes after the administration of the preparation. Sometimes it was replaced by a negative chronotropic effect, which persisted longer and disappeared gradually (Fig. 3, b,c,d). In all the experiments of this series, the vagal effects were spontaneously restored in an order opposite to that of their disappearance. The development rate and degree of the disturbances and the restoration of the parasympathetic effects specifically depended upon the amount of Methinone introduced into the blood: the changes caused by the smaller doses were slower to develop and less complete, and restoration occurred more rapidly than with the administration of the larger doses. In these experiments, there was usually no change in the contractility of the heart.

In the control experiments, in which similar amounts of a Ringer's solution were (3 ml) introduced into the frog's blood, neither the activity nor the parasympathetic effects of the heart were affected.

The results obtained showed that 2-methylnaphthoquinone possesses peculiar cholinolytic properties and that it depresses the effect of the parasympathetic nerves on the heart. However, the disturbances in the parasympathetic effects caused by Methinone are not due to a decrease in the excitability of the vagus nerves or to a decrease in

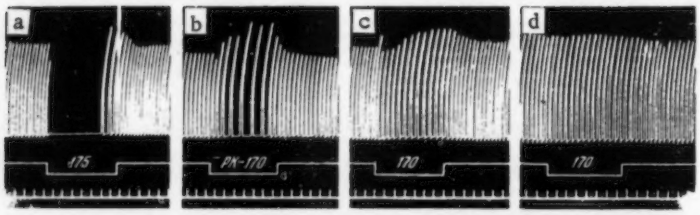


Fig. 3. Change in the parasympathetic effects after the intravenous injection of Methinone. a) Before injection; b, c, d) 5, 10, and 40 minutes respectively after the injection. Curves the same as in Fig. 1.

the heart's sensitivity to acetylcholine. In special experiments, we found that Methinone in a concentration of 1 : 50,000, which causes the gradual disappearance of the parasympathetic effects on an isolated heart, does not alter the sensitivity of the heart to pharmacological acetylcholine.

The literary data on the anti-choline-acetylase activity of 2-methylnaphthoquinone suggest that the explanation of the results obtained is disturbance of the acetylcholine metabolism and liberation of acetylcholine in the parasympathetic nerve endings. The results of the experiments with low concentrations of 2-methylnaphthoquinone acting on an isolated heart indicates that their effect is specific and is not attended by any change in the muscular elements.

The research on the effect of 2-methylnaphthoquinone permits certain conclusions about the physiological significance of acetylcholine in the parasympathetic innervation of the heart. The experiments described above demonstrated that the vagal effects are depressed in a definite order with the action of Methinone: the inotropic component is the first to disappear, followed by the vagal arrest of the heart and, lastly, by the chronotropic effect. This sequence evidently reflects the degree to which acetylcholine participates in the development of these effects. It is interesting that the changes caused by atropine in the effect of the vagus on the heart occur in an opposite order [4].

On one hand, the material obtained confirms the fact that the mechanisms of the inotropic and chronotropic effects of the parasympathetic nerves on the heart and the degree to which the chemical factor, acetylcholine, participates in these mechanisms differ considerably [5, 6, 8, 11, 12]. On the other hand, however, our experiments showed that acetylcholine plays a specific part in the formation of the chronotropic effect and, particularly, the cardiac arrest. We believe the hypothesis can be expressed that acetylcholine is not the direct cause for the development of inhibition in the automatic center in the case of arrest and a negative chronotropic effect, but only promotes the effect of the excitatory, electro-ionic component of the parasympathetic pulses; acetylcholine to some extent determines the direction and stability of the polarization changes, which are evidently caused by inhibition of the "automatism" center [7, 11, 12, 13 et al.]. In our opinion, acetylcholine plays the same role in the latter case as in the elements of the somatic nervous system [ 1, 2, 3].

The effect of higher concentrations of Methinone on the heart must be evaluated somewhat differently. The disturbance we observed in the automatism and the function of the conduction system could be caused by more profound disorders of the acetylcholine metabolism. There are data which indicate that acetylcholine plays an important part in the origin of cardiac automatism and in the function of the conduction system [9, 14, 15 et al.]. However, we are inclined to think that the disturbance of cardiac activity caused by high concentrations of Methinone is due not only to disturbance in the acetylcholine metabolism, but to the non-specific, alterative effect of 2-methylnaphthoquinone as well, which is further confirmed by the clearly expressed depression of myocardial contractility observed with the action of high concentrations.

## SUMMARY

When acting upon the isolated heart or administered into the blood flow, concentrations of 2-methylnaphthoquinone (1 : 50,000-1 : 100,000) provoke a decrease and disappearance, first of the inotropic component of the parasympathetic effect, then the vagal arrest, and, lastly, the inhibition of chronotropic component. These changes are caused by the disturbance of the acetylcholine metabolism and the liberation of this substance in the nerve endings of the vagus. Besides, large concentrations (1 : 10,000) render a nonspecific altering effect on the cardiac elements.

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* The spontaneous restoration of the vagal effects, sometimes observed with the action of weak Methinone solutions (1 : 100,000) on an isolated heart and always observed with the introduction of Methinone into the blood, seems to be due to a gradual decrease in the active concentration of the vitamin as a result of its fixation by protein substances [16].

† Original Russian pagination. See C.B. Translation.

# THE MECHANISM OF INSULIN'S TOXIC EFFECT ON A FROG'S HEART

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The works of many researchers [4, 5, 8, 12, 13] have established that the administration of toxic doses of insulin leads to significant changes in the activity of the cardiovascular system (fall of blood pressure, weakening of cardiac contractions, changes in the electrocardiographic indices, etc.) A series of authors [see 5, 12, 13] consider the results obtained to be a consequence of myocardial injury.

The data presented in the literature do not reveal the intimate mechanism of insulin's negative effect on the action of the heart. The literature does not contain any information on the biochemical structures which take part in the processes of insulin's toxic effect on myocardial activity, nor is the role of the sulfhydryl groups of the protein bodies known.

Recently, much material has been accumulated which indicates the importance of sulfhydryl groups in the chemism of muscle contraction [2, 6, 11], specifically, in the contractile function of the myocardium [6, 7, 9, 10]. For example, the works of Kh. S. Koshtoyants [6], T. M. Turpaev [10] and other authors have shown that blockade of the tissue sulfhydryl groups by thiolic poisons (cadmium chloride) causes sharp depression of the amplitude of the contractions of an isolated frog's heart. Under these conditions, the administration of cysteine ( a donator of sulfhydryl groups) or urea, which has the ability to break up the protein molecule and thereby liberate the tissue sulfhydryl groups, restores the contractile function of the myocardium.

We expressed the hypothesis that toxic doses of insulin may affect the activity of the tissue sulfhydryl groups of the protein bodies of the myocardium. In order to prove this proposal experimentally, we decided to conduct a series of experiments which would demonstrate whether the negative effect of insulin on the myocardium can be removed by the action of urea, a substance which can liberate reserve sulfhydryl groups.

## METHOD

The experiments were performed on frog's hearts isolated according to Shtraub. The heart action was recorded mechanographically and electrocardiographically. The electrocardiograms (ECG) were recorded with the aid of an EKP-4 electrocardiograph. We used depolarized electrodes ($Zn + ZnSO_4$) with cotton wicks to lead off the action currents of the heart. One electrode was placed on the region of the sinus venosus, the other, at the apex of the heart. We tested the effect of insulin in a concentration of 400 units per 100 ml of solution and the effect of urea in a dilution of $1 : 10^{-2}$. All the experimental preparations were prepared in Ringer's solutions.

## RESULTS

In all 22 experiments, the introduction of insulin into the perfusate of the isolated frog's heart caused a rapid and considerable decrease in the amplitude of the cardiac contractions. In 14 experiments, the amplitude of the cardiac contractions was only 16-30% of the original value. The subsequent 3-minute washing out of the ventricle of the heart with a Ringer's solution did not restore normal cardiac activity, but the administration of urea under these conditions caused the amplitude of the cardiac contractions to increase in all the experiments. In 12 experiments, the amplitude of the cardiac contractions was fully restored to the original level, and in 10 experiments, the amplitude was 6-14% lower than before the introduction of insulin into the heart (Fig.1).

The action of insulin on the heart caused typical changes in the ECG. To begin with, the P-Q interval, increased, although the time of the passage of excitation through the auricles did not change. The sharpest changes were observed in the QRS complex, which increased considerably in duration. The R wave became wide, acutely deformed and cloven. In most cases, the T wave dropped or became negative. The changes in the Q and S waves were not stable.

The ECG data, therefore, clearly indicates that the introduction of toxic doses of insulin causes considerable disturbances in the function of the heart's conduction system, primarily affecting atrioventricular and intraventricular conduction.

Washing out the ventricle of the heart with a Ringer's solution usually did not help restore the ECG indices of a frog's heart poisoned with insulin. After the administra-

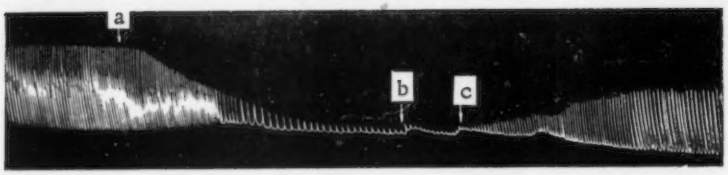


Fig. 1. Effect of urea on the contractions of an isolated frog's heart under conditions of insulin intoxication. a) Introduction of insulin; b) 3-minute washing out of ventricle of heart with a Ringer's solution; c) introduction of urea ($1 \cdot 10^{-2}$).

tion of urea under these conditions, the P-Q interval became shorter and returned to normal. The duration of the QRS complex began to decrease, indicating improved intraventricular conduction. The original direction of the T wave was restored, as was, in most cases, its original amplitude (Fig. 2).

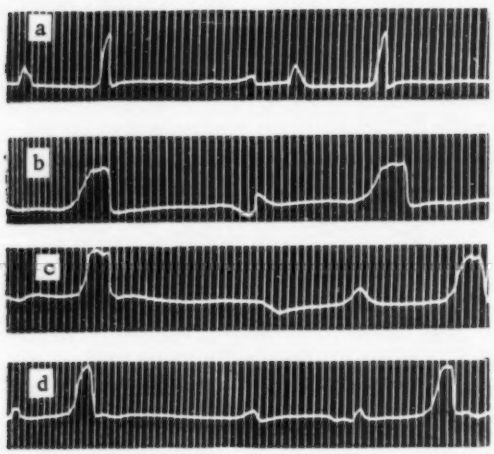


Fig. 2. Change in the ECG of a frog's isolated heart under the influence of urea administered on a background of insulin. a) Normal ECG of the isolated heart; b) ECG one minute after the action of insulin; c) ECG after 3-minute washing out of heart ventricle with a Ringer's solution; d) result of urea's effect after two minutes.

The mechanographic and electrocardiographic data obtained allow the conclusion that insulin has a considerably stronger effect on the function of the atrioventricular node and the intraventricular conduction system of the heart than on the contractile ability of the myocardium.

On the basis of the data obtained, we believe it possible to express the hypothesis that the negative effect of toxic doses of insulin on myocardial contractility and bio-electric activity may be due to a certain extent to depression of the activity of the tissue sulfhydryl groups of the protein molecules. This proposal is based on the fact that, under conditions of acute disturbance of myocardial contractility and bio-electric activity, the administration of urea helps restore these indices to normal.

The explanation of this effect evidently lies in the fact that urea, which a series of authors [1, 3, 6, 7, 10] believe to possess the ability to break up the protein molecule and liberate the tissue sulfhydryl groups, brings about the restoration of the disturbed metabolic processes in the myocardium. As indirect corroboration of this notion, we offer the fact that the changes which we observed insulin to cause in the ECG are very like the data obtained by K. S. Logunova and E. Z. Kipershlak [7] in their study of the ECG of a frog's isolated heart under conditions of cadmium chloride blockade of the sulfhydryl groups.

The opinion we have expressed as to the character of insulin's negative effect on the heart is in no way an attempt to simplify the nature of the problem by reducing the mechanism of this substance's toxic effect to change in the activity of the tissue sulfhydryl groups. Our experimental results rather suggest that the change in the reactivity of the sulfhydryl groups is of great significance in the mechanism of insulin's toxic effect on a frog's heart. Further research is needed to approach an understanding of the nature of the biochemical changes in the myocardium which occur with the action of toxic doses of insulin.

## SUMMARY

The author suggests that the negative effect of toxic doses of insulin on cardiac activity is possibly caused by depression of the activity of the tissue sulfhydryl groups. To obtain experimental verification of this suggestion, the author studied the action of urea on the work of the isolated frog's heart under conditions of insulin intoxication. Following the arrest of the cardiac activity caused by toxic doses of insulin, urea administration helped restore the contractile myocardial function and the ECG indices. Such an effect is evidently due to the fact that urea, which has the ability to loosen the protein molecule and liberate the tissue sulfhydryl groups, brings about the restoration of disturbed processes in the cardiac muscle.

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# THE EFFECT OF NEUROPLEGIC AGENTS ON RAT'S LIVER ALKALINE PHOSPHATASE ACTIVITY UNDER CONDITIONS OF BURN SHOCK

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(Presented by V. N. Chernigovskii, Active member of the AMN SSSR)

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According to modern pathophysiological theory, shock cannot be considered only a circulatory disturbance. Soviet researchers [1, 2, 3, 6, 7 et al.] were primarily responsible for the discovery that the nervous system in general and the higher nervous activity in particular play a decisive role in the development of traumatic shock. In shock, the neurohumoral regulation is disturbed, upsetting the equilibrium of almost all the vital processes in the organism and causing thereby serious disturbances of the metabolic processes [4, 17, 21]. The liver's importance in the metabolism suggests that it is involved in these pathologic processes. According to F. Engel, the liver sustains hypoxic injuries even during the early stages of shock. Engel and other authors believe that the increased blood sugar content observed in shock is due to intensified decomposition of liver glycogen. According to the observations of D. Cordier and M. Touze [see 13], the rate of glycogen phosphorylation in rat's liver decreases considerably in traumatic shock, and the inorganic phosphorus content increases. G. Le Page discovered that, under conditions of shock, there is a greater decrease in the adenosine triphosphate of the liver than in that of the other organs; the data of P. F. Minaev, show that the administration of ATP prevents the development of shock.

A series of laboratory-experimental and clinical data indicate that neuroplegic agents (derivatives of the phenothiazine group) alleviate the course of traumatic and hemorrhagic shock, as does artificial hibernation [9, 21, 15, 16, 18 et al.]. However, the question of the changes caused in the metabolism by the administration of phenothiazine preparations (chlorpromazine) or artificial hibernation under conditions of shock has not yet been solved.

On this background, we belived it expedient to study the changes in the alkaline phosphatase activity in liver tissue under conditions of burn shock, in the first place, and in the second place, to what extent these changes are affected by the administration of chlorpromazine or a hibernation mixture under the same conditions.

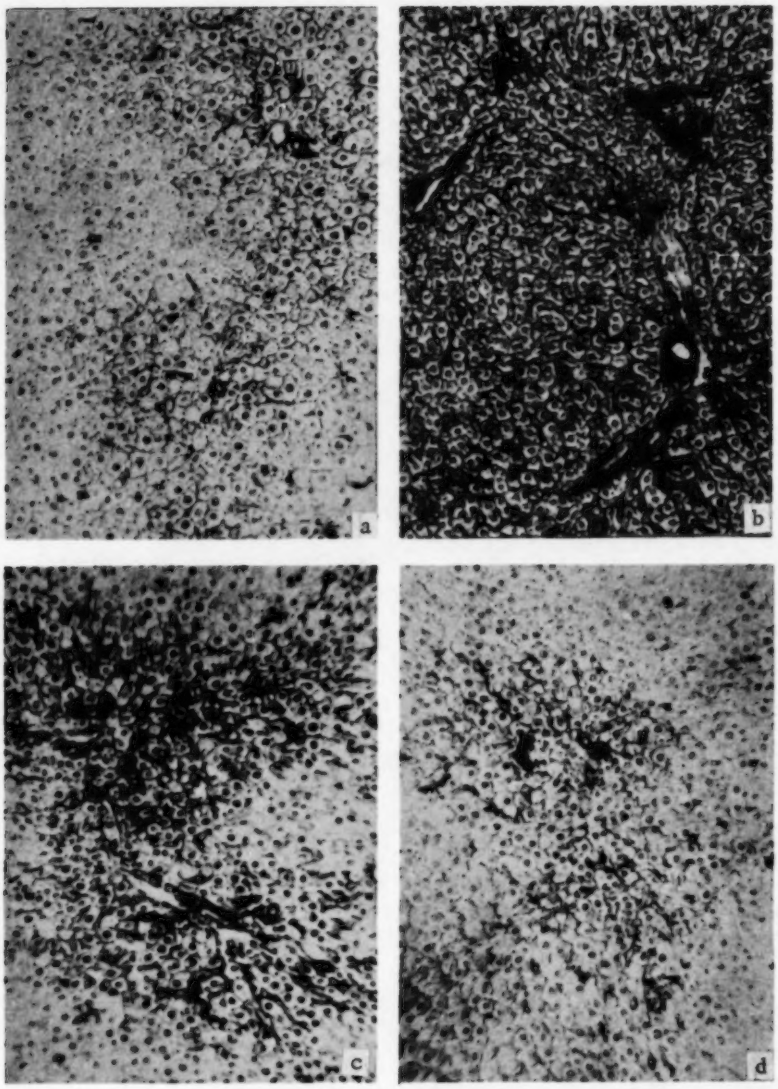
## METHOD

Experiments were performed on 42 male white rats weighing 100-120 g each. Shock was induced by the infliction of a burn, for which purpose 36 of the animals were dipped into boiling water (65°) for ten seconds; 12 of the animals which received the burn were not given preliminary treatment. Forty minutes before the infliction of the burn, 12 animals were subcutaneously injected with chlorpromazine (Largactil preparation) in a dose of 20 mg/kg and 12 animals, with the hibernation mixture (20 mg/kg Largactil + 20 mg/kg Phenergan [promethazine] + 40 mg/kg Dolantin [Demerol]). After the injection, the animals were kept at room temperature (18-20°). The temperature of the animals (taken rectally) was 20-22°. The remaining six animals, which did not receive either the burn or an injection, served as the control. The animals were killed six hours after the burn. Pieces of the liver were fixed in cooled alcohol. The alkaline phosphatase activity was demonstrated according to Gomori's histochemical method. The sections were incubated for 6 and 12 hours.

## RESULTS

The rats which had not received the preliminary treatment showed symptoms of acute excitation, then became quiet and sluggish, and their respiration was shallow and rapid. The treated animals were quite and drowsy after the burn.

We observed strongly increased alkaline phosphatase activity in the liver of the animals which had been burned without preliminary treatment (Figure). Well expressed enzymatic activity was noted throughout the liver lobes, in the bile capillaries and cell nuclei of the liver and even in the cytoplasm of the cells. The activity of the Kupffer's cells and the endothelial cells of the sinus increased to a like degree. In the animals which had received chlorpromazine before the burn, there was much less increase in the alkaline phosphatase activity. The bile capillaries, nuclei of the hepatic cells and Kupffer's cells only remained more or less active in certain parts of the sections. The phosphatase activity of the liver of the animals which had received the hibernation mixture before the burn shock hardly differed from the liver phosphatase activity in the control experiments: scattered active Kupffer's cells were observed, and the bile capillaries




Activity of rat's liver alkaline phosphatase. a) Normally; b) with burn shock; c) with burn shock after preliminary administration of chlorpromazine; d) with burn shock after preliminary artificial hibernation. Reaction according to Gomori's method. Magnification: ocular 7, objective 10.

and nuclei of the hepatic cells were somewhat more active than normally.

It should be mentioned that, in our previous investigations, the administration of these substances had no particular effect on the normal enzymatic activity of the liver.

The increased liver phosphatase activity observed after the burn shock indicates a process of intensive dephosphorylation of the substances. This corresponds to the literary data and throws new light on the process causing the loss of high-energy phosphoric acid esters. An increase in the activity of alkaline liver phosphatase has been observed in other cases besides the shock condition, for example, under conditions of hypothermia, anoxemia and the administration of formalin. A. Meier [20] observed the same phenomenon, attended by loss of liver glycogen, under conditions of starvation. D. Cordier and M. Touze [13] discovered that phosphorylation of liver glycogen is retarded in traumatic shock. One can propose that retardation of the phosphorylation process must be attended by acceleration of the dephosphorylation process.

The mechanism of the protective effect exerted by chloropromazine and artificial hiberantion with respect to the increase in alkaline phosphatase activity is not yet sufficiently clear. E. Ciocatto and his coworkers [11] and Anselmo da Cruz et al. observed that Largactil decreases

the activity of blood serum phosphatase and cholinesterase. We believe that what occurred in our experiments, however, was not a peripheral effect acting directly on the enzyme, but a central effect excluding the reactivity of the autonomic nervous system. This is indicated by the fact that while these agents do not affect normal phosphatase activity, Largactil and artificial hibernation, in toxic injury of the liver induced by chloroform, do not decrease, but even increase alkaline phosphatase activity.

## SUMMARY

The activity of the alkaline phosphatase of rat liver was found to be sharply increased under conditions of burn shock. This rise was considerably inhibited by largactyl and almost completely by the hybernation mixture (largactyl + phenergan + dolantin). The authors consider that the mentioned effect is central in origin and may play a certain role in the antishock effect of artificial hybernation.

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*Original Russian pagination. See C. B. Translation.

*ONCOLOGY*

# SPECIFIC ANTIGENS FROM TUMORS AFTER PROLONGED CULTIVATION ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO

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Research by many workers has shown that tumors of man and animals possess a complex antigenic constitution. Besides the antigens common to both normal and tumor cells [5, 6, 7, 10], the latter also possess antigens specific to tumors [1, 2, 8, 9]. Insufficient attention has been paid to the study of changes in the antigenic composition of tumors during heterotransplantation; for this reason we still have no information on the fate of individual antigens composing the tumor cell when the tumor is grafted into an animal of a different species. The solution of this problem is important for the establishment of the relationship between the blastomogenic activity and the various antigens present as components of the tumor.

We have shown that not all tumor antigens are directly related to its blastomogenic properties [4]. The problem of the role of other antigens could only be solved experimentally, and was the object of the present investigation.

## METHOD

Crocker's sarcoma of mice and M-1 sarcoma of rats were cultivated on the chorioallantoic membrane of the developing chick embryo and fully preserved their blastomogenic properties in relation to their original hosts. Back transplantation of the tumors was successful in 100% of cases

We obtained immune sera in rabbits against Crocker's mouse sarcoma and its heterotransplants on the membrane, and also against M-1 sarcoma of rats and organs of normal rats, by a method previously described by us [7]. Immune sera were also obtained in White Leghorn fowls against tissues of Crocker's sarcoma and its first transplantation on the chorioallantoic membrane. Immunization was carried out as follows: on the first, third and sixth days the fowls were injected with 1 ml of a saline extract of the tissue intravenously, and thereafter three times with 2 ml of the extract intraperitoneally, at intervals of four days. Blood samples were taken from an incision of the comb on the seventh day after the last injection of antigen. To obtain sera, the fowls were exsanguinated on the 9th-10th day.

In order to detect the specific tumor antigen in M-1 sarcoma and its heterotransplantates, the immune rabbit sera were tested by the complement fixation reaction at + 37°. To study the tumor antigen in Crocker's sarcoma, however, it was impossible to use this reaction, for during immunization of rabbits with an extract of Crocker's sarcoma antibodies were produced not only to the tumor antigen but also to the heterogenic antigen common to the chorioallantoic membrane of the chick embryo; as we have shown, this antigen is a constituent of the Crocker's sarcoma cell [3]. For this reason, in the investigation of the sera before absorption by this means, it was impossible to differentiate between tumor and membrane. It was also impossible to free the immune sera against Crocker's sarcoma from antibodies against the membrane by means of the absorption reaction, because these antibodies did not arise nonspecifically in the antitumor sera, but were true, immune antibodies.

In the study of the tumor antigen of Crocker's sarcoma, we therefore investigated the sera without specific absorption, and by the precipitation reaction.

The antigens used in the reactions were saline extracts of the tissues to be tested. The antigens for the precipitation reaction were prepared in a ratio of tissue to physiological saline of 1 : 20. In addition, these antigens were centrifuged for 30 minutes at 10,000-13,000 rpm. The precipitation reaction was read at 20 minutes.

## RESULTS

In Table 1 we give the results of a comparative study of the antigenic properties of a Crocker's mouse sarcoma and of its heterotransplants on the chorioallantoic membrane.

It can be seen from Table 1 that the sera of fowls immunized with a saline extract from the first transplantation of the Crocker's tumor (Nos. B-7210, B-7214, and B-3269) reacted from only with antigens from tumors grown on the membrane, but also with antigens from mouse tissues, and completely failed to react with antigens from normal chorioallantoic membrane. This indicated an antigenic difference between the cultivated tumor and the membrane, and also that the antigen used for the immunization, i.e., the first transplantation of Crocker's tumor on the chorioallantoic membrane of the chick embryo, was foreign in relation to the fowl.

TABLE 1. Comparative Study of the Antigenic Properties of Crocker's Sarcoma and of its Heterotransplants on the Chorioallantoic Membrane of the Chick Embryo

| Serum no. | Animal from which serum was obtained | Results of precipitation reaction with tissue antigens from | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Crocker's sarcoma | mouse liver | mouse spleen | normal chorio-allantoic membrane | Crocker's sarcoma after transplantation | | | | | | | | mouse serum |
| | | | | | | once | 10 times | 11 times | 22 times | 23 times | 24 times | 26 times | 28 times | |
| Not numbered, nonimmune | Fowl | — | — | — | — | | — | — | | | | | | — |
| B-7210, against 1st transplantation | » | + | + | + | — | + | | | | | | | | — |
| B-7214, against 1st transplantation | » | + | + | + | — | + | | | | | | | | — |
| B-3269, against 1st transplantation | » | + | + | + | — | + | + | + | | | | | | — |
| B-0921, against Crocker's sarcoma | » | + | + | + | — | + | | | | | | | | — |
| B-0997 " " | » | + | + | + | — | + | | | | | | | | — |
| B-0923 " " | » | + | + | + | — | + | + | + | | | | | | — |
| 691 | Rabbit | + | + | + | — | | | | + | + | + | + | + | + |
| 248, against mouse serum proteins | » | + | + | + | — | — | — | — | — | — | — | — | — | + |
| 254, against normal membrane | » | — | — | — | + | | — | — | | | | | | — |
| 255, against normal membrane | » | — | — | — | + | | | | — | — | + | + | + | — |

Sera against the original Crocker's tumor, also obtained in fowls (Nos. B-0921, B-0997, and B-0923) and rabbits (No. 691), reacted with mouse tissue antigens and with antigens from the allantoic tumors, and thus indicated an antigenic relationship between them.

Immune sera against Crocker's tumor and the first transplantation of the tumor on the membrane were not specific antitumor sera, for they reacted with normal mouse tissues; consequently, the antigen which we discovered in the chorioallantoic tumors could not be described as a specific tumor antigen. This cytoplasmic antigen bore the species properties of the mouse, although it was not identical with mouse serum proteins, for the precipitating serum No. 248, reacting with a species-specific antigen identical with the serum proteins, did not react with antigens from transplanted tumors, although it did react with a species-specific antigen in extracts obtained from mouse tissues.

Antisera against the normal chorioallantoic membrane of the developing chick embryo reacted only with antigens from the membrane and with antigens from a few transplanted tumors, which could be accounted for by the presence of membrane in the allantoic tumors in the capacity of stroma.

The serum of a nonimmune fowl, taken as a control, did not give a positive precipitation reaction with any of the antigens mentioned, and it thus followed that normal fowl serum did not contain antibodies to these tissues.

It could thus be shown by means of the sera investigated that Crocker's sarcoma retains the antigens inherent in the original tumor even after prolonged cultivation on the chorioallantoic membrane of the chick embryo. This common factor is independent of the presence of a heterogenic antigen, and also of a species-specific antigen identical with the serum proteins, in the original tumor and especially in the tumors grown on the chorioallantoic membrane. This species-specific antigen, as we have shown earlier [4], is lost by the tumor during heterotransplantation.

The results of the study of the specific tumor antigen in the M-1 sarcoma of the rat are shown in Table 2.

It can be seen from the results in Table 2 that immune sera against M-1 tumors (Nos. 169 and 773) were specific antitumor sera, and reacted only with M-1 rat sarcoma and with its heterotransplants on the chorioallantoic membrane of the chick embryo, while failing to react with normal rat tissues and normal chorioallantoic membrane. The sera thus detected a specific tumor antigen in heterotransplants of the tumors on the membrane and demonstrated the absence of an antigenic relationship between the M-1 sarcoma and the chorioallantoic membrane. Serum No. 145 against M-1 sarcoma was less specific, but it too did not react with antigen from the membrane, and it reacted more strongly with tumor antigens than with antigens from normal rat tissues. Sera against normal organs of the rat reacted neither with M-1 sarcoma nor with its heterotransplants, but reacted specifically with antigens from the particular organ against which they were obtained. Immune serum No. 254 against normal chorioallantoic membrane detected the presence of

TABLE 2. Specific Tumor Antigen in Heterotransplants of M-1 Sarcoma

| Serum no. | Dilution of serum | Results of complement fixation reaction with tissue antigens from | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M-1) sarcoma | rat liver | rat spleen | normal chorio-allantoic membrane | M-1 sarcoma, after transplantation: once | twice | 3 times | 4 times | 5 times | 6 times | 7 times | 8 times | 9 times | 10 times |
| 169, against M-1 sarcoma | 1:60 | ++++* | – | – | – | ++++ | ++++ | ++++ | | ++++ | +++ | ++++ | ++++ | ++++ | +++ |
| | 1:80 | +++ | – | – | – | +++ | +++ | +++ | | +++ | ++ | +++ | +++ | +++ | +++ |
| | 1:100 | +++ | – | – | – | ++ | +++ | +++ | | +++ | ++ | +++ | ++ | +++ | ++ |
| | 1:120 | +++ | – | – | – | + | +++ | +++ | | +++ | ++ | +++ | ++ | +++ | + |
| 773, against M-1 sarcoma | 1:20 | ++++ | + | – | – | ++++ | ++++ | ++++ | ++++ | +++ | ++++ | | | | |
| | 1:40 | ++++ | – | – | – | ++ | +++ | ++++ | +++ | +++ | ++++ | | | | |
| | 1:80 | +++ | – | – | – | ++ | ++ | ++ | ++ | +++ | ++ | | | | |
| | 1:160 | +++ | – | – | – | + | + | – | – | ++ | ++ | | | | |
| 145, against M-1 sarcoma | 1:60 | ++++ | +++ | ++ | – | ++++ | ++++ | +++ | ++++ | ++++ | +++ | ++++ | +++ | ++++ | ++++ |
| | 1:80 | ++++ | +++ | ++ | – | ++++ | +++ | +++ | ++++ | +++ | ++ | ++++ | ++ | ++++ | +++ |
| | 1:100 | ++++ | ++ | + | – | ++++ | +++ | ++ | ++++ | +++ | ++ | ++++ | ++ | ++++ | +++ |
| | 1:120 | ++++ | ++ | – | – | ++++ | ++ | + | ++++ | +++ | ++ | ++++ | ++ | ++++ | +++ |
| 1058, against rat liver | 1:40 | – | ++++ | – | – | – | – | | | – | – | – | – | – | – |
| | 1:80 | – | ++++ | – | – | – | – | | | – | – | – | – | – | – |
| | 1:160 | – | ++++ | – | – | – | – | | | – | – | – | – | – | – |
| | 1:320 | – | ++ | – | – | – | – | | | – | – | – | – | – | – |
| 128, against rat spleen | 1:40 | – | – | ++++ | – | – | – | | | – | – | – | – | – | – |
| | 1:80 | – | – | +++ | – | – | – | | | – | – | – | – | – | – |
| | 1:160 | – | – | ++ | – | – | – | | | – | – | – | – | – | – |
| | 1:320 | – | – | + | – | – | – | | | – | – | – | – | – | – |
| 254, against normal chorio-allantoic membrane | 1:40 | – | – | – | ++++ | | | | | ++ | ++ | ++++ | ++ | +++ | ++++ |
| | 1:80 | – | – | – | ++++ | | | | | ++ | ++ | ++++ | ++ | +++ | ++++ |
| | 1:160 | – | – | – | ++++ | | | | | ++ | ++ | +++ | + | ++ | +++ |
| | 1:320 | – | – | – | ++++ | | | | | + | + | + | + | + | +++ |

* The complement fixation reaction was assessed as ++++ if complete fixation of complement too place: as +++, ++ and + if different degrees of hemolysis were observed, and by a minus sign if hemolysis was complete.

the antigen of the chorioallantoic membrane of the chick embryo in the tumor heterotransplants, which evidently indicated the inclusion of membrane into the stroma of the allantoic tumors.

It can thus be seen from these cross reactions (see Table 2) that heterotransplants of M-1 sarcoma, like the original M-1 sarcoma, possess a specific tumor antigen, differing from the antigens of normal rat tissues and of normal chorioallantoic membrane.

Our results thus showed that Crocker's sarcoma of mice possesses, in addition to the two antigens previously described, a third or cytoplasmic antigen, bearing the species properties of mice, which is evidently a tumor antigen that is preserved in spite of prolonged heterotransplantation of the tumors in a foreign environment.

The species-specific antigen identical with mouse serum proteins disappeared after only the first transplantation of the tumor on the chorioallantoic membrane, although the tumor did not lose its blastomogenic properties in relation to the original host. The heterogenic antigen, common to Crocker's sarcoma and the chorioallantoic membrane, was characteristic of normal tissues (mouse spleen, chorioallantoic membrane) besides the tumor, but is absent from tumors · such as Ehrlich's adenocarcionoma [3] and M-1 sarcoma of rats, and it cannot therefore be responsible for the blastomogenic properties of tumor tissue.

The specific tumor antigen, preserved in heterotransplants of M-1 sarcoma of rats, and the third, cytoplasmic antigen, bearing the species properties of mice, which is evidently a tumor antigen also, were detected in heterotransplants of tumors which had retained their properties of producing tumors in the original hosts.

These facts give grounds for attributing a definite connection with the tumor-producing properties of the tumors to this biochemical antigenic complex.


## SUMMARY

The authors studied the specific tumor antigen in Crocker's sarcoma of mice and M-1 sarcoma of rats as well as in heterotransplants of these tumors on the chorioallantoic membrane of the developing chick embryo. It was shown that this antigen was preserved in the heterotransplants of these tumors. The possible connection of preservation of the specific tumor antigen with the retention of the blastomogenic properties of the heterotransplants with regard to the initial hosts of the tumor is discussed.


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# A COMPARATIVE STUDY OF THE ACTION OF A LIVING CULTURE OF BACILLUS PRODIGIOSUS (SERRATIA MARCESCENS) AND ITS PRODUCTS ON THE GROWTH OF A BROWN-PEARCE TUMOR IN RABBITS

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From sources in the literature we know that certain bacterial toxins (filtrates and lysates of bacterial cultures) and their polysaccharide-containing fractions have the power to depress the growth of malignant tumors in animals, or even of causing their complete absorption. It has been shown, in particular, that a bacterial fraction (Shear's "polysaccharide"), isolated from a culture of Bacillus prodigiosus, causes lasting hemorrhagic changes and necrosis in muscle sarcomas and other tumors [7, 11, 13, 14, 16-19]. Physicochemical methods have demonstrated that such fractions are not only polysaccharide in nature, but consist of a lipo-proteino-polysaccharide complex or of bacterial endotoxin, possessing well-defined antigenic properties [5, 6, 8, 10, 12, 15].

The problem of the antitumor activity of a living culture of B. prodigiosus has received comparatively little study. In isolated researches [4], and also in our own investigations [1, 2, 3], it has been shown that a living culture of this microorganism sharply depresses the growth of sarcomas of rats and, in the majority of cases, leads to the absorption of Brown-Pearce carcinoma in rabbits. Certain authors [9], however, consider that a bacterial infection produced by administration of B. prodigiosum, has no therapeutic influence on the growth of tumors in mice. According to their findings, it is not the living culture but its toxins or bacterial "polysaccharides" which possesses antitumor activity.

In this connection it was of interest to make a comparative study of the effect of living and killed cultures of B. prodigiosus, and also of its "polysaccharide" fraction, on the development of Brown-Pearce carcinoma in rabbits.

## METHOD

Experiments were carried out on sexually mature male chinchilla rabbits. The animals, which were divided into five equal groups (with five rabbits in each group), were inoculated intratesticularly with Brown-Pearce carcinoma in a dose of 0.5 ml of a 20% tumor suspension. The "polysaccharide" fraction was obtained from a culture of B. prodigiosus by the method of Perrault and Shear and of Savage [13, 18]. It contained 32.9% of protein as nitrogen and 59.3% of reducing substances, calculated as glucose. The preparation for testing possessed high toxicity towards laboratory animals. After the rabbits had been immunized with this fraction, their blood showed the presence of specific antibacterial agglutinins (against B. prodigiosus), evidence of the antigenic properties of this preparation. The bacterial endoxin used was also a culture of B. prodigiosus, which was killed by heating to a temperature of 60° for $1\frac{1}{2}$ hours. An emulsion of a living culture of this microoganism was prepared in sterile physiological saline 15-20 minutes before the beginning of its injection.

Treatment with the bacterial preparations began on the tenth day after inoculation of the tumor. The rabbits of the first group were given intravenous injections of living B. prodigiosus cells in a dose of 800 million bacterial cells per kg body weight. The rabbits of the second group were treated in the same way, but with a killed culture. The animals of the third and fourth groups received corresponding injections of "polysaccharide" fraction in a dose of 0.3-0.03 mg/kg body weight, also intravenously. Altogether, each animal received seven injections at intervals of four days. The rabbits of the fifth group acted as controls.

In the second series of experiments, 20 rabbits were used, and these animals were inoculated with Brown-Pearce tumor into the right testicle, in a dose of 0.75 ml of a 20% suspension. Six days later, the testicle together with the tumor nodule was removed. On the third day after operation, ten rabbits (experimental group) were injected intravenously with a living culture of B. prodigiosus in a dose of 900 million bacterial cells per kg body weight. The remaining injections were given intraperitoneally in a dose of $2 \times 10^9$ bacterial cells per kg body weight. Seven injections were given altogether, five at intervals of three days, and the last two at intervals of ten days. Control rabbits received injections of physiological saline along similar lines.

The efficacy of the antitumor action of the bacterial preparations was assessed by two indices: 1) The

TABLE 1. Study of the Action of Living and Killed Cultures of B. prodigiosus and of Their Fractions on a Brown-Pearce Tumor

| Group of rabbits | Preparations injected | No. of rabbits used in experiment | Outcome of the tumor | | | | |
|---|---|---|---|---|---|---|---|
| | | | number of rabbits dying | | | sacrificed after 3 months | length of survival (in days) |
| | | | up to 4th day | up to the 80th day | total | | |
| First | Living microorganisms . . | 5 | — | 1 | 1 | 4 | 91 |
| Second | Killed microorganisms . . | 5 | 3 | 1 | 4 | 1 | 59 |
| Third | Fraction (maximum dose) . . . . . | 5 | 2 | 1 | 3 | — | 41 |
| Fourth | Fraction (minimum dose) . . . . . | 5 | 3 | 1 | 4 | 1 | 55 |
| Fifth | Control . . . . . | 5 | 5 | — | 5 | — | 32 |

Note: In assessing the results of the experiment we ignored two rabbits (of the third group) which died after the first injection of the "polysaccharide" fraction.

TABLE 2. Results of Counts of the Metastases in the Organs of Rabbits after the Action of a Living Culture of B. prodigiosus and its Products on a Brown-Pearce Tumor

| Group of rabbits | Preparations injected | No. of rabbits used in the experiment | Outcome of the tumor | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | number of rabbits | | metastases | | number of metastases | | number of organs affected | |
| | | | dying | surviving | present | absent | total | per rabbit | total | per rabbit |
| First | Living microorganisms. | 5 | 1 | 4 | 4 | 1 | 46 | 11,5 | 17 | 4,3 |
| Second | Killed micro. . . | 5 | 4 | 1 | 5 | — | 672 | 134,4 | 42 | 8,4 |
| Third | Fraction (maximum dose) . . | 5 | 3 | — | 3 | — | 1 517 | 505,6 | 29 | 9,6 |
| Fourth | Fraction (minimum dose) . . . | 5 | 4 | 1 | 5 | — | 675 | 135,0 | 31 | 6,2 |
| Fifth | Control . . . . . | 5 | 5 | — | 5 | — | 2394 | 478,8 | 60 | 12 |

Note: In assessing the results of the experiment we ignored two rabbits (of the third group) which died after the first injection of the subtoxic dose of the "polysaccharide" fraction.

TABLE 3. Effect of a Living Culture of B. prodigiosum on the Survival of Rabbits Inoculated with Brown-Pearce Carcinoma after Removal of the Primary Tumor

| Group of rabbits | No. of rabbits used in the expt. | Outcome of the tumor | | | | | |
|---|---|---|---|---|---|---|---|
| | | number of rabbits dying | | | sacrificed after three months | survival rate, % | mean length of survival (days) |
| | | up to the 35th day | up to the 80th day | total | | | |
| Experimental | 10 | 1 | 2 | 3 | 7 | 70 | 80,6 |
| Control | 10 | 7 | 1 | 8 | 2 | 20 | 46,8 |

TABLE 4. The Effect of a Living Culture of B. prodigiosus on Metastases of a Brown-Pearce Carcinoma after Surgical Removal of the Primary Tumor

| Group of rabbits | No. of rabbits used in the expt. | Outcome of the tumor: number of rabbits | | metastases | | number of metastases | | number of organs affected | |
|---|---|---|---|---|---|---|---|---|---|
| | | dying | sacrificed after 3 mo | present | absent | total | per rabbit | total | per rabbit |
| Experimental | 10 | 3 | 7 | 5 | 5 | 413 | 82,6 | 25 | 5 |
| Control | 10 | 8 | 2 | 10 | 0 | 6 644 | 644,4 | 103 | 10,3 |

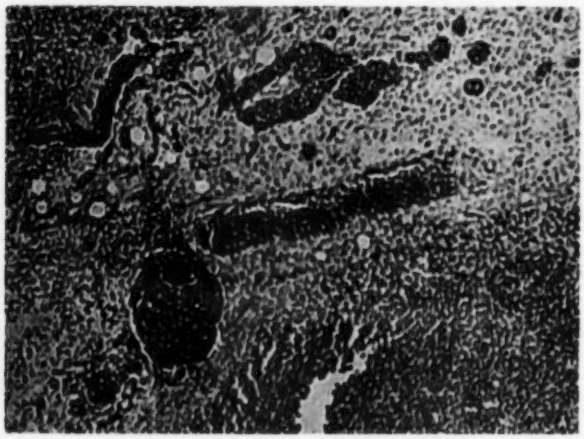

Fig. 1. Brown-Pearce carcinoma of the testicle in a rabbit. Hemorrhage and destructive changes in the tumor tissue after intravenous injections of the "polysaccharide" fraction of the microorganism (obtained by Shear's method). Magnification: ocular 6 ×, objective 8×, coefficient 1.4.

Fig. 2. Brown-Pearce carcinoma of the right testicle in a rabbit. Hemorrhagic necrosis of the tumor after intravenous injections of the "polysaccharide" fraction of the microorganism (obtained by Shear's method).

survival rate of the animals (the rabbits were kept under observation for three months, and were then sacrificed); 2) the degree of metastasization of the tumor.

## RESULTS

The results of the experiments are shown in Tables 1, 2, 3, and 4.

It can be seen from Table 1 that the course of development of the tumor differed in each group of animals. All the control rabbits were dead on the 35th-40th day after inoculation of the tumor. In the group in which a living culture was used, no deaths were observed among the animals during this time. In the second group, only one rabbit survived until the day of sacrifice, and of the remainder, three died on the 40th and one on the 80th day from inoculation of the tumor.

After the injection of a subtoxic (third group) or tolerated (fourth group) dose of "polysaccharide" fraction, we observed no therapeutic action. The majority of animals, as in the second and control groups, died at earlier stages of development of the tumor.

The longest period of survival was thus observed in the animals injected with the living culture. Only one rabbit of this group died up to the 80th day from inoculation of the tumor, and all the rest survived and were in good condition throughout the three months of observation.

At postmortem examination of all the rabbits dying or sacrificed after three months, we found that tumor metastases had developed in nearly all the animals. It can be seen from Table 2 that the largest number of metastases and of organs affected was observed in the fifth (control) and the third groups of rabbits, in which the length of survival was least. In the second and fourth groups of rabbits the degree of metastasization was approximately the same. Nevertheless, the number of metastases in the control was $3\frac{1}{2}$ times greater than in these animals.

In the first group, despite the high virulence of the inoculated tumor, its degree of metastasization was the lowest. In this group of animals, development of the tumor was shown by the appearance of only solitary (in two or three organs) small metastases with signs of degeneration and absorption.

In connection with the results obtained it is of interest to study the morphological picture of a tumor subjected to the action of the bacterial preparations. We were interested in particular with the animals injected with the different doses of the "polysaccharide" fraction of the microorganism. Histological investigation showed that in the majority of such rabbits some degree of hemorrhage and degenerative and necrotic changes developed in the tissue of the testicular tumor (Fig. 1). In individual cases (in three of the eight rabbits after the second intravenous injection of the preparation) an extensive zone of hemorrhagic necrosis of the testicular tumor was formed, which extended to the subcutaneous cellular tissue and the skin of the scrotum (Fig. 2). Despite such a severe reaction on the part of the tumor vessels, however, metastases developed as a rule in the rabbits and led to the death of 80-100% of the animals.

The results of our investigation fully confirm the earlier findings of Shear and others [17-19] that the polysaccharide-containing fraction of B. prodigiosus does, in fact, cause hemorrhagic changes in the tumors of experimental animals. At the same time, it follows from our results that the intravenous injection of a living culture of B. prodigiosus into rabbits with a Brown-Pearce carcinoma, inoculated intratesticularly, is more effective in its antitumor action than the injection of a heat-killed culture or of its "polysaccharide" fraction. A therapeutic effect was also found from the action of a living culture of this microorganism on the tumor in the second series of experiments.

It may be seen from Table 3 that the majority of animals in the control group (80%) died before the time of sacrifice: seven on the 30th-35th day and one on the 80th day after inocualtion of the tumor. In the experimental group at this time only three rabbits had died, and the remaining seven survived throughout the three months of observation in a good condition.

A longer period of survival was thus observed in the animals treated with the microorganisms.

The results of postmortem examination (Table 4) show that the control and experimental rabbits differed significantly from each other by the degree of metastasization of the tumor.

In the control group, carcinoma nodules were found not only in the rabbits which died, but also in two rabbits sacrificed after 3 months. In one of these, for instance (No. 2261), an enormous tumor of the left eye and one of the wall of the stomach (dimensions 1.5 × 1.5 cm) were found, together with a tumor of the adrenal gland and multiple metastases in the lungs, the large and small intestine and the kidneys. In the other rabbit (No. 2157), the tumor had developed less intensively than in the remaining control animals, although metastases in the kidneys were also observed in this animal. It should be mentioned that in these two rabbits the majority of the metastases were in a state of regression, although among them were some recent, growing tumor nodules.

In five of the seven surviving rabbits in the experimental group no metastases were present in the organs, but in two we observed residual signs of a tumor in the form of scars on the surface of the parenchymatous organs or of pigmented spots (for example, on the serous membrane of the large intestine and the parietal peritoneum). In the experimental rabbits which died, a relatively high degree of metastasization was observed. Nevertheless, considerably fewer metastases were found in these animals than in the dying control animals.

On the basis of the experiments described above, it may be concluded that the intravenous and intraperitoneal injection of a living culture of B. prodigiosus into rabbits with Brown-Pearce carcinoma, inoculated intratesticular-

ly, leads to absorption of the primary tumor and to suppression of metastasization. The results of the experiments fully confirmed our previous findings [1] that a living culture of B. prodigiosus possesses an antitumor action.

## SUMMARY

A polysaccharide containing fraction was isolated from B. prodigiosus; when injected intravenously this fraction provoked hemorrhagic changes in a Brown-Pearce tumor inoculated intratesticularly in the rabbit. However, the living culture of this microorganism (administered by the same method) possessed a more effective-suppressive effect on the growth of Brown-Pearce tumors than the culture killed by heating, or its "polysaccharide" fraction.

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# THE DEVELOPMENT OF BONE SARCOMA IN RATS INJECTED WITH STRONTIUM-89 AND THE EFFECT OF STRONTIUM-89 ON THE OFFSPRING OF SUCH RATS

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The study of the effect of radioactive isotopes on living animals and, in particular, on the development of bone tumors, and also the consequences of this effect are problems of great importance at the present time. The literature of recent years dealing with the study of the bone changes and the formation of bone tumors by the action of radioactive isotopes contains only a limited number of reports, and some of these are concerned with the study of strontium-90.

Our investigations are devoted to the study of the action of strontium-89, differing essentially from strontium-90 by its half-life period: Strontium-89 has a short half-life period of 54.5 days, whereas strontium-90 is characterized by a comparatively long half-life period, lasting 19.5 years.

The data in the literature on this subject includes changes in bone tissue under the influence of the "bone" isotopes during both the acute and chronic periods [1-14]. All the authors cited conclude that strontium, when it enters the body, is deposited in the bones, where a process of pathological reorganization of the tissue is initiated, as a result of which, after a certain time, sarcomas of bone develop. The number of sarcomas differed, according to the findings of these authors, and varied between 30 and 70%. These figures, however, relate mainly to strontium-90.

## METHOD AND RESULTS

Strontium-89 was injected intraperitoneally into rats in aqueous solution, in a dose of 1 $\mu$C/g body weight.

For the experiments 156 rats were used; in 78 of these animals neoplasms of bone were observed, and in 60 of these the tumors were single and in 18 multiple. Altogether 102 tumors developed. Histological investigation showed that these were osteomas and osteogenic sarcomas. In addition we investigated the bones of the offspring of the rats which received injections of strontium-89 (in the first and second generation) for their strontium content.

The largest number of tumors was situated in the femur (42) and tibia (38). Significantly fewer neoplasms developed in other parts of the skeleton. In the humerus, for instance, only eight neoplasms were found; the same number of tumors was present in the bones of the vertebral column. There were five tumors in the bones of the pelvis and only one in the bones of the forearm.

The tumors appeared at the following times: Minimum – 157 days, maximum – 461 days. The highest mortality among the animals was observed in the seventh and eighth months (32 and 47 rats). In the same period (from the sixth to the ninth month), one of the signs of radiation sickness – depilation of the rats – was most pronounced.

A sharp decrease in the number of young in the litters was observed in many females, especially in the first six months after injection of strontium: Instead of 10-12 in each litter, there were only four or five; only later, one year after the injection of strontium, was the number of young rats in the litters once again raised to normal.

To two rats, 52 and 69 days after the beginning of the experiments, eight young rats were born in two litters. Six of these had developmental defects of the bony skeleton: In all of them the tail was hardly developed, and in two, in addition, a severe maldevelopment of the hindlimb was observed (Fig. 1).

Among the rats of the second and third generations, individuals were also encountered in which the tail was shortened, and in one animal in the second generation there was maldevelopment of the hindlimb. This particular rat was born 449 days after the start of the experiment.

Regarding the indications given in the literature concerning the deposition of strontium in the bones, we were interested in the question whether a connection existed between the localization of strontium in the bones and the site at which the tumors developed. In order to answer this question we used the method of autoradiography. Sections of bone from the experimental animals were placed on x-ray film and, after exposure for a period from 15 minutes to five days, the film was developed; as a rule radiation tracks could be seen on it, the intensity of which depended on the amount of strontium in the section. In the course of this work we tried various exposure times. In N. N. Litvinov's work [2, 3, 4] with strontium-90, the

Fig. 1. Rat with maldevelopment of the limb and tail (experimental treatment with $Sr^{89}$).

Fig. 2. Autoradiograph of the femur of a rat receiving $Sr^{89}$. Intensive blackening in the cortical layer of the diaphysis. Exposure 30 minutes.

most intensive impressions were obtained with an exposure lasting several days, and the maximum exposure reached 20 days; in our experiments the optimum time for production of a clear autoradiograph was only 30 minutes. According to our films, the strontium was localized as a rule in the cortical layer of the bones, in the region of the diaphysis, so that darkening of the x-ray film took place more intensively in the area corresponding to the diaphysis than in that corresponding to the metaphysis (Fig. 2). The majority of neoplasms arose in the diaphysis. This suggests that the foci of development of sarcomas coincide with the sites of greatest localization of strontium.

In addition to the bone sections of rats which had received injections of strontium, we also prepared a series of bone sections from young rats of the first generation and, by increasing the exposure times to 1-5 days, we obtained very clear autoradiographs of these sections. This indicated that the bones of the first generation of young rats also contained strontium. Tests with a $\beta$-radiation counter confirmed the activity of the young rats' bones and, consequently, the presence of strontium therein. This made it necessary to discover how the strontium passed from the mother to the young. Two ways may be postulated: The first – through the placenta, in the period of intrauterine development, and the second – through the milk. Theoretically, either of these methods of transmission of strontium is permissible, and it is noteworthy that in the literature there are indications of activity of the milk.

We took from the suckling rats receiving strontium-89 a small quantity (about 0.5 ml) of milk and tested its activity. Not one of three samples of milk showed any activity. A few drops of blood were taken from these same rats, and the activity determined. In every case the blood tests showed definite activity, although admittedly only very slight. Moreover, the very fact of the birth of rats with developmental defects of the bony skeleton itself suggests the placental route of transmission of strontium from mother to fetus during the period of intrauterine development. The absence of strontium from the milk in our observations may, to some extent, be accounted for by the considerable length of time elapsing from the start of the experiment to the day of the test (about 200 days). In order, however, to obtain final confirmation of the role of the placental route, we tested the activity of the femur in a newborn rat which had not yet begun to feed on its mother's milk. The result of the test was positive – activity was established, and the autoradiograph gave an obvious impression after an exposure of five days (Fig. 3).

These findings all go to prove that strontium-89 passes from mother to offspring via the placenta.

Fig. 3. Autoradiograph of the femora of newborn rats. Exposure 5 days.

During tests of activity, we found radiation in a living rat of the first generation, and also in the bones of the rats of both first and second generations. This fact suggests that the influence of radioactive substances may be spread not only to the animal receiving injections of strontium, but also to the offspring, and even to the second generation.

## SUMMARY

$Sr^{89}$ was administered intraperitoneally to rats. In 50% of those which survived for 180 days and longer bone sarcoma appeared (mainly in the femur and tibia), and some of them developed multiple tumors. The tumors were most commonly revealed 8-9 months after the beginning of the experiment. The mortality rate, as well as the loss of hair in the rats which developed no tumors, was greatest at the same periods of time (i.e., during the 8th-9th month). The progeny of rats treated with $Sr^{89}$ (the first and the second generation) was usually born with skeletal anomalies; the autograms of these animals showed the presence of strontium. By checking the activity of milk and blood it was established that $Sr^{89}$ passes from the mother to the progeny through the placenta.

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*EXPERIMENTAL BIOLOGY*

# SPLEEN REGENERATION IN MICE FOLLOWING REMOVAL OF THE MAJOR PART OF THE ORGAN

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Despite the fact that regeneration of the spleen of mammals has been studied since the middle of the XIX century, there is still no unified opinion about its capacity for restoration following partial resection [1, 2, 5, 6, 7]. In our laboratories É. I. Mun'kina and G. I. Sol'ts [4] and B. M. Levitina [3] observed the regeneration of the spleen in certain mice and rats after the removal of half the organ However, a detailed description of the structure of the regenerating organ was not given in these works.

In an attempt to eliminate the indicated shortcoming we set up experiments on the regeneration of the spleen in mice, removing the major part of the organ (80-90%), since the study of a small fragment of the spleen makes it easier to acquire impression of the structure of the regenerating organ.

## METHOD

The removal of 80-90% of the organ was performed on 160 white pubertal male mice, 15-18 g in weight. Both ends were amputated from the externalized spleen, leaving in the middle, at the site of vascular entry, a piece 1-2 mm in width. The portion of the spleen removed was weighed. In order to gain an impression of the weight of the fragment remaining in the animal's body, a piece of the removed portion of the spleen was cut out, approximately equal to that fragment in its dimensions. We will subsequently arbitrarily call the weight of this piece the original weight of the retained fragment. This piece was fixed and subjected to histological study. Each mouse had its marking, so that it was possible to know how much the residual fragment weighed (on the basis of the weight of a piece comparable to it) as well as the weight of the spleen regenerating from it.

The animals were sacrificed by decapitation on the 2nd, 4th, 6th, 14th, 21st, 28th, 38th, and 73rd days after the operation. Normal mice were sacrificed on the 14th, 28th, 38th, and 73rd days at the same time as the experimental animals, these serving as controls. Upon the death of the animals several of the spleens were fixed in Zenker's solution, imbedded in paraffin, and the sections stained with hemalaun plus eosin. In these preparations the number of mitoses and Malpighian bodies were counted. The average measurement of the Malpighian body was 350-400 $\mu$, and thus they were counted in every 50th section (width of the section was 7 $\mu$). The mitoses were counted in a center section across a Malpighian body, using 100 visual fields (ocular 10×, objective 90 ×; diapragm in the ocular with a quadratic aperture measuring 7 × 7 mm).

For a clearer outlining of the white pulp from the red, several regenerating spleens were fixed in 12% formalin and treated by the method of Lepene (histochemical reaction to peroxidase). Longitudinal and cross sections of the spleens were prepared; 5-10 sections, taken from each piece, were drawn on paper, using the Edinger apparatus, noting by different shading the parts corresponding to the white and red pulp. Then the pieces corresponding to the white and red pulp were cut out and seperately weighed and the ratio between the areas that they occupied computed.

Data on the measurements and weighings of the spleens and remaining fragments are presented in Tables 1 and 2. They give evidence of the fact that during the period of observation regeneration processes took place in the residual fragments.

Two days after the operation the weight of the residual fragment increased by an average of two times. Up to that time, as can be seen from Table 3, the ratio of the area occupied by the white pulp to the area occupied by the red was less than in the normal; this is apparently evidence of the increased blood storage of the fragment. In all fragments there were observed processes of destruction, manifested to a varying degree.

Further increase in the regenerating fragment was observed after 4-6 days. At this time the processes of break-down had somewhat diminished. Proliferation was noted in the reticular tissue, and the processes of myelopoiesis, lymphopoiesis and erythropoiesis were proceeding intensively. The dimensions of the small Malpighian bodies increased to a certain degree. The ratio of the area of the white and red pulps was equal to 0.74 (one fragment examined), i.e., was within normal limits. Beginning with two weeks, in the majority of animals there was observed such significant increases in the dimensions and weights of the organs that it was already possible to decide with certainty in which cases regeneration occurred.

At a different time of the year conflicting results were obtained. Thus when the experimental animals

TABLE 1. The Average Weight of the Fragment and of the Spleen Regenerating from It at Different Intervals After the Operation

| Duration of the expt. (in days) | Total no. of animals in the series | Number of animals in which regeneration occurred | Original wt. of the spleen (in mg) | Wt. of the residual fragment of spleen at time of operation (mg) | Weight of the regenerated spleen (mg) |
|---|---|---|---|---|---|
| 2 | 6 | 6 | 76,0 | 9,5 | 18,2 |
| 4 | 5 | 5 | 84,2 | 11,2 | 23,4 |
| 6 | 6 | 6 | 82,5 | 10,3 | 41,1 |
| 14 | 13 | 10 | 104,0 | 11,4 | 42,0 |
| | 10 | 8 | 83,0 | 8,7 | 28,3 |
| | 21 | 9 | 114,0 | 9,4 | 27,7 |
| 21 | 7 | 5 | 53,7 | 6,2 | 30,0 |
| 28 | 14 | 11 | 53,0 | 7,3 | 43,1 |
| | 12 | 10 | 95,7 | 10,5 | 49,7 |
| 38 | 25 | 21 | 64,4 | 5,0 | 42,8 |
| 73 | 10 | 10 | 86,0 | 10,0 | 54,1 |

TABLE 2. The Average Dimensions of the Fragment Remaining After Operation and of the Regenerated Spleen

| Length of expt. (days) | Size of residual spleen fragment (in mm) | | | Size of regenerated spleen (in mm) | | |
|---|---|---|---|---|---|---|
| | length | width | thickness | length | width | thickness |
| 2 | 4,0 | 2,0 | 1,5 | 4,4 | 2,9 | 2,0 |
| 4 | 4,4 | 2,1 | 1,3 | 4,6 | 3,4 | 2,0 |
| 6 | 4,1 | 1,9 | 1,1 | 6,3 | 3,8 | 2,8 |
| 14 | 4,6 | 2,1 | 1,3 | 5,1 | 3,3 | 2,3 |
| 21 | 3,5 | 1,7 | — | 4,4 | 3,3 | — |
| 28 | 4,6 | 2,1 | — | 5,7 | 4,6 | — |
| 38 | 4,7 | 1,1 | 1,7 | 6,1 | 4,0 | 2,7 |
| 73 | 4,8 | 2,1 | 1,9 | 6,0 | 4,5 | 3,8 |

were sacrificed in the spring, after 14 days, regeneration of the spleen was observed in two different series in 80 and 77.7% of all cases, while after the same number of days in the fall, the residual fragment of spleen increased in only 43.4% of the mice; in the remaining animals during the elapsed two week period the fragment decreased or underwent complete destruction. The weight of the regenerated fragment two weeks after the operation increased by an average of 3 to 3 $^1/_2$ times. In one animal the regenerated fragment of spleen exceeded the weight of the entire organ on the day of operation: 31 mg in place of 30 mg.

TABLE 3. The Ratio of the Area Occupied by the White and Red Pulp in Sections Across the Spleen

| Residual fragment of spleen | Duration of regeneration | |
|---|---|---|
| | 2 days | 14 days |
| 0,99 | 0,81 | 0,52 |
| 0,91 | 0,81 | 2,09 |
| 0,71 | 0,89 | 0,74 |
| 0,98 | 0,68 | 0,79 |
| 0,74 | 0,65 | 0,50 |
| 0,86 | 0,70 | 0,81 |
| 0,85 | — | 0,90 |
| 0,86 | — | 0,83 |
| 0,98 | — | 1,21 |
| 0,97 | — | 0,92 |
| 0,91 | — | 1,29 |
| 0,96 | — | 1,51 |
| 1,11 | — | — |
| 0,96 | — | — |
| 0,72 | — | — |
| 0,75 | — | — |
| 0,89 | 0,76 | 1,01 |

In cross sections it was possible to observe a significant increase in the residual fragment; the sections did not have a triangular form, but rather a round shape, the angles being rounded out. The longitudinal sections were

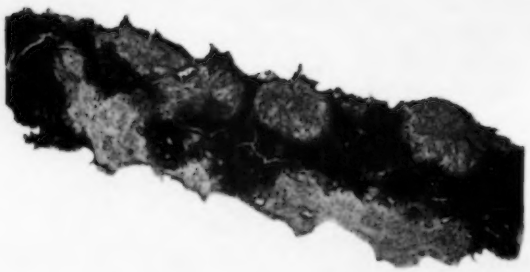

Fig. 1. Longitudinal section of the fragment of spleen remaining after operation. Magnification: ocular 5×, objective 4×. Stained according to the method of Lepene.

Fig. 2. Longitudinal section of a regenerated spleen 14 days after the operation. Magnification: ocular 5×, objective 4× Stained according to the method of Lepene.

especially indicative, in which the mass of the regenerated fragment was markedly increased (Fig. 1 and 2), as was the reticular stroma and the number of Malpighian bodies.

A count of the number of Malpighian bodies showed that two weeks after the operation the number increased by 2 $^{1}/_{2}$ times in comparison with the control. In the original fragment the number of Malpighian bodies on the average (14 animals) was equal to 8.9, while in the regenerated fragment it reached 22.4 (8 animals).

The number of mitoses in the Malpighian bodies per 100 visual fields also increased significantly in comparison with the control: from 8 (6 animals) to 31 (4 animals).

It was possible to note 3 types of interrelationships between the red and white pulps in the regenerated spleens. In several animals the ratio of the area of the white pulp to the area of the red did not differ from that characteristic for the controls; in others there was observed a considerable increase in the area occupied by the white pulp; in 2 animals the ratio of the area of white and red pulp was less than in the control.

It was possible to note further increase in the weight of the regenerated spleens 3-4 weeks after the operation. In several series the weight of the spleen increased by 4-5 times. However, the proliferative processes at this time had quieted down somewhat. The number of mitoses 3 weeks after the operation decreased to 12.5 (average for 4 mice).

Upon sacrificing the experimental animals 38 days after the operation regeneration of the organ was observed in 84% of the cases. In 4 mice the weight of the regenerated organ considerably exceeded the weight of the spleen at the time of operation. However, the average weight of the regenerated spleens (76, 104, 68, and 86 mg in place of 53, 54, 55, and 70 mg respectively) at this time had still not attained the weight of the spleens in the control animals, which was equal to an average of 160 mg.

Sacrifice of the animals 73 days after operation showed that the fragment of spleen increased by an average of 5 times; in several cases it reached the weight of the spleen in the control mice. The ratio of the area of white and red pulps was equal to 1.1.

The regenerated fragments of spleen and the spleen of the control mouse are presented in Fig. 3. The weight

Fig. 3. Fragments of spleen (a), remaining after operation (weight 6 and 9 mg); regenerated spleens (b) 73 days after operation (weight 82 and 97 mg); spleen (c) of the control animal (weight 95 mg).

of the regenerate was 97 mg, the original fragment being 6 mg; the length of the regenerate was 6 mm, of the original fragment, 1 mm. The original form of the organ was not reconstructed; the fragment increased evenly in all three measurements. The ratio of the white and red pulps in this fragment was equal to 0.95.

The data obtained bears evidence of the capacity of the spleen of mice for regeneration after removal of 80 to 90% of the organ. This may be adjudged on the basis of the marked increase in the weight of the spleen. Histological investigation showed that the increase in the weight of the fragment of spleen is not caused by blood engorgement, but by an increase in the mass of the organ's parenchyma. The ratio of the mass of the white and red pulp characteristic for the normal was preserved in the regenerating spleen, and the number of Malpighian bodies increased; the latter, however, did not reach the normal, which indicates a certain hypertrophy of the Malpighian bodies in the course of regeneration.

Our observations permit, to a certain degree, the explanation of the conflicts present in the literature on the question of the capacity of the spleen for regeneration. As is shown by the data presented, regeneration of the spleen does not occur in all cases, and, at different times of the year, it is manifested to different degrees. It is also possible that several authors denied the capacity of the spleen for regneration on the basis of the fact that they did not account for the peculiar means of its reconstruction, wherein along with well manifested regeneration phenomena the original form of the organ is not reconstructed (regeneration hypertrophy).


## SUMMARY

White mice were subject to spleen amputation from both ends, with a fragment thereof left intact, equal to about 1/8 to 1/10 of the initial weight of the organ. A considerable increase in the weight of the spleen was noted in the mice sacrificed 14 to 73 days after the operation. In individual instances the weight of the regenerating spleen equalled that of the spleen of control mice. The ratio between the area occupied by the white and the red pulp approached normal. The number of Malpighian bodies was increasing but would not reach their initial level. The spleen failed to recover its original shape, i.e., its restoration pursued the course typical for regenerative hypertrophy.


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# ON THE "GLYCOGEN FUNCTION" OF THE MYOMETRIUM IN PREGNANCY

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In a preceeding investigation [1] it was shown that with the advent of pregnancy there is a gradual increase in the amount of glycogen in the muscles of the uterus, attaining a maximum at the time of birth. The physiological significance and the mechanism of regulation of this process remained unelucidated, however, since it was not successfully connected with changes in the contractile activity of the myometrium [2].

In subsequent investigations [3] it was shown that in the regulation of the "glycogen function" of the myometrium essential importance is apparently held by impulses from the fetal side, developing in the uterine cavity. Thus, it was shown that if animals (female rabbits) in which the fallopian tube on one side was ligated, became pregnant, as a result of which the fetuses developed in only one horn of the uterus, then in the mucosal and muscular layers of this "empty" horn glycogen was not accumulated, at the same time that its quantity in the horn containing the fetuses was able to reach 4-5% in relation to the dry weight of the tissue [1, 3].

The data of these experiments provided a basis for postulating that the process of glycogen accumulation in the myometrium during pregnancy holds some sort of meaning in the development of trophics of the intrauterine fetus.

The task of the present investigation involved further study on the question of the possible physiological importance of the "glycogen function" of the myometrium and its regulatory mechanisms.

## METHOD

The experiments were carried out on female rabbits. The peritoneal cavity was opened under novocaine anesthesia. The horn of the uterus was quickly detached and on a refrigerated frosted glass opened lengthwise along the mesometrial side. The fetuses and placentae were removed, and the amputated periplacental portions of the horn of the uterus were frozen with dry ice to a firm state. The mucosal layer was separated from the frozen "disks" of tissue by means of a scalpel. The solid portions of uterine muscle were immersed in a warm solution of potassium hydroxide, after which the amount of glycogen in them was determined by the method of Pflueger. The result of each investigation was presented as the arithmetic mean of 2-3 parallel determinations.

For the investigation of the rate of separation of the tissue glycogen during its incubation the unfrozen portion of uterine tissue was suspended in a glass container, 50 ml in volume, with a ground stopper. On the bottom of the container there was filter paper moistened with water. In this moist chamber the tissue was kept in an incubator at 37° for 1 hour. The tissue was then frozen, treated according to the description above, and the amount of glycogen in the muscular layer, remaining after an hour of incubation, determined. The intensity of the separation of glycogen during the incubation was expressed in percent of its original amount. In a number of the experiments after the incubation the amount of glycogen in the tissue increased somewhat. It is necessary to postulate that in those cases a technical error took place in the separation of the mucosal layer.

The operation of removing one or several fetuses was performed under novocaine anesthesia, or, rarely, under total ether narcosis. The fetus was carefully removed through a small longitudinal incision in the wall of the horn on the antimesometrial side, along with its sacs and all the perifetal waters. A ligature was placed about the umbilical cord of fertilized eggs and drawn tightly. The fertilized egg was removed, the end of the umbilical cord together with the ligature embedded in the cavity of the uterus, and the wound closed with a continuous suture. The rapid and careful performance of the operation permitted removing one or several fetuses, without causing exfoliation of their placentae and without disrupting the normal development of the remaining fetuses in the intact fetal receptacles of the operated and unoperated horns of the uterus.

## RESULTS

The results of several of the experiments, pertaining to 14-20 days of pregnancy, are presented in Fig. 1. These experiments showed that in the interlocular portions, i.e., in the portions of the myometrium between the isolated fetal receptacles, there was contained practically the same amount of glycogen as in the myometrium of the non-pregnant animals (about 100 mg %), while in

Fig. 1. The concentration of glycogen in the different portions of uterine muscle depending on the duration of pregnancy. a) In the periplacental portions; b) in the interlocular portions.

the myometrium of the fetal receptacles of the same uterine horn glycogen was contained in quantitites several times greater. It must be noted that such irregularity in the distribution of glycogen is found in animals with a multiple pregnancy. In pregnant women there are no marked differences in the concentration of glycogen of the uterine muscles as compared to the muscle of the lower segment [2].

The data presented in Fig. 1 confirms the already stated contention that the accumulation of glycogen in the myometrium during pregnancy is related, apparently, to the life-activity of the fetus developing in the cavity of the uterus. It was essential to obtain, as far as possible, direct indications of the presence of such a relationship. For this purpose, 1-2 fetuses were removed from one horn of the uterus on the 14th to 19th day of pregnancy. On the 18th to 20th day of pregnancy, using novocaine anesthesia, the peritoneal cavity was opened and portions of the intact and operated uterine horns were taken for determinations of the amount of glycogen in the myometrium. The portions of the uterus corresponding to the site of removal fetuses were easily discovered by the traces of the suture lines (the same incision was cut to take tissue for the investigation). Signs of inflammation or adhesion were not detected in a single instance.

The results of the investigation performed are presented in the table. They show that in the myometrium of the portions of the horn in which fetuses were removed there is a lower concentration of glycogen than in the myometrium of the intact horn containing fetuses. However, it is clearly seen that the fewer the days passed between the removal of a fetus and the determination of the glycogen, the smaller this difference: It was completely absent after an interval of 3 days, it was insignificant after an interval of 4-5 days, and it was markedly manifested after an interval of 14-15 days. It is very probable that this is related to the presence or absence

The Concentration of Glycogen in the Myometrium of the Intact and Operated Horn Following the Removal of Fetuses

| Animal No. | Removal of the fetus (day of pregnancy) | Glycogen determination (day of pregnancy) | Day after operation | Concentration of glycogen (in mg%) | |
|---|---|---|---|---|---|
| | | | | intact horn | operated horn |
| 1 | 15-th | 18-th | 3-rd | 233 | 236 |
| 2 | 14-th | 18-th | 4-th | 279 | 257 |
| 3 | 17-th | 21-st | 4-th | 363 | 326 |
| 4 | 18-th | 23-rd | 5-th | 274 | 250 |
| 5 | 19-th | 24-th | 5-th | 336 | 262 |
| 6 | 16-th | 30-th | 14-th | 1 138 | 167 |
| 7 | 15-th | 30-th | 15-th | 964 | 463 |

of the "living" functioning placenta. The experiments showed that if the investigation was carried out 5-6 days after the operation, the placenta in the portions of the myometrium between the fetal receptacles from which a fetus was removed remained thickly implanted in the uterine wall and retained the usual "living" appearance. If the investigation was carried out 14-15 days after the operation, the placenta was shown to be freely moveable in the cavity of the uterus, and considerably altered in regard to size, color, and consistency. Not excluded, however, is the possibility that the importance is held not by the presence or absence or a functioning placenta, but the interval of time elapsed from the instant of removal of the fetus: After 3-5 days the absence of a fetus may still not express itself, while after 14-15 days it is expressed in a completely defined fashion. In any case, the data presented in the table shows that the fertilized egg, developing in the uterine cavity, regulates the "glycogen function" of the myometrium.

In the process of investigating the concentration of glycogen in the uterus it was discovered that in definite functional states of the uterus the glycogen contained in its muscular layer was characterized by unusual stability: It either did not separate or it separated to an insignificant degree after prolonged storage of the tissue. This phenomenon was studied in a series of experiments in which the intensity of separation of the glycogen during incubation of the isolated rabbit uterine tissue was determined. Since the original quantity of glycogen in one experiment was sometimes 10-15 times greater than in another, it was necessary, in special experiments, to clarify whether or not the detected difference in the intensity of separation of the glycogen depended on the difference in its original quantity. These experiments showed that there was no direct dependency between the intensity of separation of the glycogen and the original amount of glycogen in the tissue.

Fourteen experiments were set up on non-pregnant rabbits. Of these, in 11, after one hour of incubation, no

glycogen remained in the uterine muscles – it separated completely. Only in 3 trials was there still discovered about 1/3 of the original quantity. After a duration of pregnancy of from 12 to 17 days (5 experiments) after 1 hour of incubation an average of 62 ± 2.5% of the glycogen was broken down in the muscles of the uterus. In none of the experiments was the glycogen completely broken down, as was observed in the myometrium of the non-pregnant uterus. After a pregnancy of from 18 to 28 days (12 experiments) after 1 hour of incubation an overage of 17 ± 5.3% of the original quantity of glycogen was separated from the muscles of the uterus, while in 5 out of the 12 trials its quantity practically did not change throughout the time of incubation. Since the difference between the mean proportions characterizing the separation of the glycogen in the myometrium of the isolated non-pregnant uterine tissue in the first and second half was shown to be statistically valid, it is possible to consider it proven that with the advent of pregnancy and the increase in its duration, the "stability" of the glycogen in the myometrium increases.

At the very end of pregnancy, for 1-2 days before parturition, separation of the glycogen during the period of incubation was practically absent in 5 out of the 6 trials. In only one trial did the amount of glycogen decrease after 1 hour of incubation by 18%. Thus, at the end of pregnancy there is observed a further increase in the "stability" of the glycogen in the myometrium. In 5 experiments the intensity of separation of the glycogen in the uterine muscles of the rabbits was determined at the time of parturition, and in 18 experiments, in rabbits during the first 48 hours after parturition. The data obtained is subjected to analysis with difficulty, in view of the great scattering of the values. It is possible to postulate that this is related to the rapid and difficultly measured changes in the functional state of the uterine muscles during parturition and shortly after parturition. In any case, in the post-partum period the separation of glycogen after incubation of the uterine muscle is already marked by absolute clarity, attaining an average of 39 ± 6.3% and consisting, in isolated experiments, of even 54-74% of the original values.

Figure 2 affords the opportunity of comparing the data obtained in these experiments. It shows how significantly and quickly the "stability" of the myometrial glycogen increases along with the development of the pregnancy.

It is appropriate to mention Wertheimer's investigation [4] at this time, which showed that the glycogen in the liver of the fetus and in the placenta also possesses a high degree of stability: It does not separate in the presence of such powerful glycogen-mobilizing actions as the introduction of adrenalin, hunger, or generalized chilling of the organism, as well as in the presence of tissue autolysis.

The results presented in the present experimental investigation show that in the regulation of the "glycogen

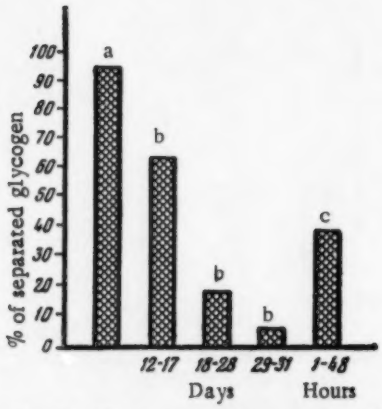


Fig. 2. The intensity of separation of the glycogen during incubation of the myometrium (mean data). a) Outside pregnancy; b) during pregnancy (indicated days of the pregnancy); c) after parturition (indicated hours).

function" of the myometrium an essential role is played by impulses associated with the life activity of the fertilized egg developing in the uterine cavity, the nature of which is still not elucidated.

The character of the distribution of the glycogen in the myometrium, and specifically its accumulation in large quantities in the fetal receptacles and its almost complete absence in the interlocular portions of the uterus, point to the influence of these impulses on the metabolism of the myometrium localized within the boundaries of one uterine horn or even within the boundaries of one fetal receptacle.

Apparently, it may be considered proven that such a clearly expressed physiological reaction of the myometrium to pregnancy as the accumulation in it of large amounts of glycogen is regulated by the fetus developing in the uterine cavity. This makes highly probable the hypothesis that the physiological importance of this "glycogen function" of the myometrium is connected with the necessities of the developing fetus and, apparently, with its trophics.

## SUMMARY

Investigation was made relative to the physiological significance of the "glycogen function" of myometrium in pregnancy and of the mechanisms regulating it. The amount of glycogen in the sections of myometrium lying between individual fetuses was found to be same as in those of non-gravid animals. The glycogen level in myometrium directly bordering on the fetuses was several times greater. The glycogen level in the myometrium of the uterine horn from which the fetuses were removed 3-5-15 days prior to the investigation was lower than in the myometrium of the intact horn containing fetuses. With the development of pregnancy there is a growing "resistance" of the myometrial glycogen during its incubation in a thermostat at 37°C. This "resistance" appears to be

maximal near the term of pregnancy. The data obtained conforms with the results of previous investigations. It shows that in the control of the "glycogen function" of myometrium an important role is played by the impulses appearing in connection with the vital activity of the fetus and that the physiological significance of this myometrial function is assocated with the requirements of the developing fetus, and probably with its trophics.

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# SOME PECULIARITIES IN THE ANTIGENIC PROPERTIES OF HUMAN EMBRYONIC TISSUES WHEN CULTURED ON VARIOUS MEDIA

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The possibility of changes in the antigenic properties of tissues as a result of heterotransplantation or of growth in tissue cultures on heterogenic media has been studied by many authors. However, the data obtained has been contradictory. Thus, Korngold and Lipari [5], in their investigation of human tumor tissues which had been preliminarily been passed through rat, hamster, and chick embryos for prolonged periods, found that human tumor tissue, successfully growing in an organism of another genus, retains its antigenic properties and does not acquire new ones characteristic of the host organism. On the other hand, V. K. Kozlov [1, 2] noted that with heterotransplantation a tumor loses its original antigenic properties and acquires new ones, characteristic of the proteins in the tissues of the new host.

In studying tissue explants, growing in vitro on heterogenic media, G. V. Suvorova [4] found that with comparatively prolonged cultivation (about 3 months) the explants showed considerable reduction in their original antigenic activity. M. S. Lomakin [3] came to the same conclusions in studying the influence of heterogenic media on the antigenic composition of cultured tissues. According to his data, in the process of cultivation tissues partially lose their initial intrinsic antigenic properties and acquire the antigenic properties of the tissues of the species of animal in whose serum they are cultivated.

The task of the present work was the study of the antigenic properties of normal tissues grown in vitro on heterogenic media. Fundamental interest was directed toward clarifying the possibility that the antigenic properties inherent in human tissues are retained in conditions involving the absence of human proteins in the nutrient medium.

## METHOD

The type of material used consisted of 6-12 week old human embryos, from which tissues were taken for explantation from the extremities without the skin covering (i.e., muscular and connective tissue). The culturing was carried out over the course of 3 months in Karrel flasks using chicken plasma, and bovine embryonal extract, and serum; cultures were set up using human serum to serve as controls, while the three experimental groups were calf, rabbit, and horse respectively. The amount of serum in the fluid phase in all groups was equal to 40%. The cultures, both experimental and control, were transplanted at the same time every 14 days. The fluid phase was replaced every 7 days. Twice in each passage, 7 and 14 days after transplantation, the explants were tested for their reaction in complement fixation studies. The fixation reaction was performed with 3 guinea pig sera: 1)Serum immunized against muscle and connective tissue from the human embryo, 2) serum immunized against the muscle and connective tissue of an adult human (tissues taken from individuals who died secondary to accidental trauma), and 3) serum from healthy guinea pigs ( as controls for the sera).

Immunization of the guinea pigs was carried out in the following manner. A 10% aqueous-saline extract of the corresponding tissue (embryonal or adult) was injected into the animals, using 0.5, 0.75, 1, 1.5, 2 ml at intervals of 3 to 4 days. Seven days after the last injection the guinea pigs were bled and the obtained sera tested for its reaction in complement fixation studies using the original material. In this fashion, the titer of the sera studied was 1 : 160 to 1 : 320. The active dose of complement was titered before the main experiment in the presence of the serum of healthy guinea pigs and of antigens. The complement fixation reaction was set up with 2 doses of complement. Fixation occurred in the course of 18-20 hours at + 4°.

## RESULTS

In the process of cultivation the usual intensive growth of the human embryonic tissue was detected. The growth zone consisted mainly of fibroblasts. In external appearance the control cultures and the experimental one, i.e., the ones growing on the heterogenic sera, did not differ from each other. At the end of the 2nd to the beginning of the 3rd month there was noted a certain lag in the growth of the cultures being cultivated on the horse and calf sera. In the cultures growing on rabbit serum the intensity of the growth was the same as in the controls. The lag in rapidity of growth pointed out in the experi-

Data on the Complement Fixation Reaction of the Extracts from Human Embryonal Tissue Explants Grown on Various Sera

| Serum | Dilution | Original material | In human serum, passages | | | | | | In calf serum, passages | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 | 3 | 4 | 5 |
| Serum of guinea pigs immunized against muscle and connective tissue from human embryos | 10 | ++++ | ++++ | + | ++++ | +++ | ++++ | ++++ | ++ | ++ | ++++ | ++++ | ++++ | +++ |
| | 20 | ++++ | +++ | + | ++++ | +++ | ++++ | ++++ | ++ | + | ++++ | ++++ | ++++ | +++ |
| | 40 | ++++ | +++ | + | + | ++ | +++ | +++ | ++ | ± | ++ | — | +++ | +++ |
| | 80 | +++ | ++ | + | + | ++ | ++ | +++ | ++ | — | ++ | — | + | — |
| | 160 | + | + | + | + | — | + | — | — | — | — | — | — | — |
| | 320 | + | + | — | — | — | ± | — | — | — | — | — | — | — |
| Serum of guinea pigs immunized against muscle and connective tissue of adult humans | 10 | ++++ | +++± | + | ++++ | +++ | ++++ | ++++ | + | — | ++++ | +++ | ++++ | ++++ |
| | 20 | ++++ | +++ | — | ++++ | +++ | ++++ | ++++ | — | — | ++++ | +++ | ++++ | ++++ |
| | 40 | +++ | +++ | — | ++ | ++ | ++++ | ++++ | — | — | ++++ | +++ | ++++ | +++ |
| | 80 | ++ | ++ | — | ± | ++ | ++++ | ++++ | — | — | +++± | ++ | +++ | ++ |
| | 160 | + | ++ | — | ± | + | +++ | +++ | — | — | — | ++ | ++ | + |
| | 320 | — | — | — | — | + | ++ | ++ | — | — | — | — | — | — |
| Serum of healthy pigs | 10 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | 20 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Control antigen | I dose | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | II » | — | — | — | — | — | — | — | — | — | — | — | — | — |

Note. Duration of cultivation per passage: 0) 14 days, 1) 28 days, 2) 42 days, 3) 56 days, 4) 70 days, 5) 84 days.

Data on the Complement Fixation Reaction of the Extracts from Human Embryonal Tissue Explants Grown on Various Sera (continued)

| Serum | Dilution | Original material | In horse serum, passages 0 | 1 | 2 | 3 | 4 | 5 | In rabbit serum, passages 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum of guinea pigs immunized against muscle and connective tissue from human embryos | 10 | ++++ | +++ | ++++ | +++ | ++ | ++ | ++ | ++ | ++ | + | +++ | ++++ | ++++ |
| | 20 | ++++ | +++ | ++++ | +++ | ++ | ++ | + | ++ | ++ | — | +++ | ++++ | ++++ |
| | 40 | ++++ | ++ | ++++ | + | ++ | ++ | + | ++ | ++ | — | ++ | +++ | +++ |
| | 80 | +++ | ++ | ++++ | — | ++ | + | ± | ++ | — | — | — | ++ | — |
| | 160 | + | ++ | ++ | — | ++ | + | — | — | — | — | — | ++ | — |
| | 320 | + | + | — | — | — | — | — | — | — | — | — | — | — |
| Serum of guinea pigs immunized against muscle and connective tissue of adult humans | 10 | ++++ | ++ | ++++ | + | ++ | + | ++ | ++ | + | ++++ | +++ | ++++ | ++++ |
| | 20 | ++++ | ++ | +++ | — | + | + | ++ | ++ | — | ++++ | +++ | +++ | ++++ |
| | 40 | +++ | ++ | +++ | — | + | + | ++ | + | — | + | + | +++ | ++++ |
| | 80 | ++ | — | ++ | — | + | — | + | + | — | + | + | +++ | +++ |
| | 160 | + | — | — | — | — | — | + | + | — | + | ± | ++ | ++ |
| | 320 | — | — | — | — | — | — | — | — | — | — | ± | ++ | — |
| Serum of healthy pigs | 10 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | 20 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Control antigen | I dose | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | II » | — | — | — | — | — | — | — | — | — | — | — | — | — |

Note. Duration of cultivation per passage: 0) 14 days, 1) 28 days, 2) 42 days, 3) 56 days, 4) 70 days, 5) 84 days.

mental groups was not very great and did not hinder the serological investigations.

In studying the complement fixation reaction of the explant extracts, both experimental and control, it was established that the human embryonal tissues retain the antigenic properties inherent to human tissues throughout all intervals in the course of cultivation, independent of whether or not there is human protein present in the nutrient medium; in addition, the antigenic activity of the explants in relation to sera immunized against human tissues does not decrease in the process of cultivation. The tissues being cultured on human sera gave complement fixation reactions with immunized sera in approximately the same dilutions as the tissues grown on heterogenic media. In prolonged intervals of cultivation there was still not noted any weakening in the antigenic activity of the experimental explants in comparison with the controls (see table).

In studying the data presented in the table on the two immunized sera, the serum against the embryonal tissue and the serum against the tissue of an adult human, differences were noted in the degree of intensity of the complement fixation reactions in those sera with the same antigens at different intervals of cultivation. If the explants of the first passages yielded complement fixation in high dilutions of the antiembryonal serum, then the complement fixation in the presence of extracts from the explants of the 4th to 5th passages (2 $^1/_2$ to 3 months in vitro) occurred even with high dilutions of the serum against the tissues of the adult human. The change in the antigenic activity of the explants in regard to these sera did not evidence a strictly regular character, but, in general, was sufficently clear. In the process of explantation the tissues altered their antigenic composition, which expressed itself by a decrease in the antigenic activity in relation to antiembryonal serum, and, on the other hand, an increase in relation to the serum against the tissues of the adult human.

The indicated changes in the antigenic properties of the cultured tissues were noted in both the control and experimental groups.

Thus, in the process of cultivation human tissues retain the antigenic properties inherent to the human, although they change somewhat, partially losing antigens characteristics of embryonal tissues and acquiring antigens characteristic of the tissues of the adult human.

The possibility of explaining the given phenomenon has still not presented itself; it serves as the subject for our further investigations. It is only possible to state several hypotheses. First, the indicated phenomenon may be related to changes occurring in the embryonal tissues during its comparatively prolonged cultivation. Second, it is not here possible to exclude the influence of the nutrient media, in particular the serum, which was taken from adult animals and naturally had a somewhat different protein composition than the serum of the embryos.

## SUMMARY

The human antigenic properties are retained by the human embryonic muscular and connective tissues in culturing for the period of 3 months in homo- or heterogenic medium. The antigenic propertes of these tissues are changed during the process of culturing: There is a gradual weakening of the properties inherent to the embryonic tissues, with the intensification of these characteristic of the muscular and connective tissues of adult.

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# RESTORATION OF OVARIAN STRUCTURE IN RATS FOLLOWING DISTURBANCES CAUSED BY ORTHOAMINOAZOTOLUOL

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The introduction of cancerogenic substances into an organism causes alterations in the weight and morphologic characteristics of the sex glands. However, data dealing with this question it scarce and extremely contradictory. It was discovered [7] that in rabbits that were smeared with tar the ovaries became heavier than in control animals. The increase in weight occurred as a result of an increase in the interstitial tissue. In the presence of a tar-induced tumor there takes place in rabbits a suppression of ovarian function [8], which is expressed as an inhibition of the development of follicles. A number of authors [5, 6] caused a lowering of the fertility of mice by smearing them with tar. A decrease in the function of the ovaries ("ovarian aging") was noted [4] in mice in which coal tar was applied to the skin. The effect of subcutaneous administration of 1, 2, 5, 6-dibenzanthracine was also studied [3]. This substance caused a lowering of ovarian function in mice which was manifested by a decrease in the number of large follicles and yellow bodies. However, the use of benzopyrene [2] did not cause similar changes in the mice ovaries. Upon smearing the mice with orthoaminoazotoluol over a period of 2-4 weeks [1] a decrease in the weight and function of the ovaries was noted; with more prolonged administration of orthoaminoazotoluol the weight of the ovaries increased more than 2 times in comparison with their weight in the control animals.

In those ovaries there was noted an increase in the proliferative processes.

The purpose of the present work involved the study of alterations in the function and structure of the rat's ovaries caused by prolonged administration of orthoaminoazotoluol and the possibility of restoration following these disturbances.

## METHOD

Sexually mature rats, 198-244 g in weight, were used in the experiment. Along with its food, each experimental animal received 1 ml of a 6% solution of orthoaminoazotoluol daily in sunflower oil. Over the course of 7 months, the function of the sex glands was studied by means of the vaginal smear technique. The experimental animals, along with their corresponding control animals, were sacrificed at 1, 2, 3 $\frac{1}{2}$, and 7 months after the beginning of the experiment.

With the purpose of studying the restorative processes in the ovaries, one group of experimental rats, 10 in number, were kept under observation for an additional 5 months after the administration of orthoaminoazotoluol (for 12 months) had ceased. Then these animals were sacrificed. The left ovary was fixed in 80° alcohol for determination of the level of alkaline phosphatase by the method of Gomori [10] and of RNA by the method of Brachet [9]. The right ovary was fixed in Zenker's solution containing acetic acid for the study of the morphologic alterations and an appraisal of the structural components. For this purpose a series of sections were prepared, 7 $\mu$ in width; the preparations were stained with hematoxylin and eosin.

The structural components were counted in every 5th section of the ovary of the control and experimental rats after $3\frac{1}{2}$ and 7 months of administration of orthoaminoazotoluol, as well as in the ovaries in the restoration period following the effect of orthoaminoazotoluol. In each group the structural components of the ovaries were counted in 7-10 rats.

## RESULTS

In control animals with a normal rhythm of the sexual cycle and normal oogenesis a considerable amount of alkaline phosphatase and RNA was noted in the structural components and stroma of the ovaries. The cells of the germinal epithelium were very rich in RNA and alkaline phosphatase. Many enzymes were contained in the external, follicular, and ovarian layers of oocytes, which were in different stages of maturation. The cytoplasm and nucleoli of the follicular cells were also rich in RNA.

In the nuclei of the oocytes of primordial follicles the activity of alkaline phosphatase was high; dark-staining clumps of nuclear material were concentrated mainly next to the nuclear membrane. The nucleolus contained considerable amounts of enzyme and RNA. In the cytoplasm of the oocytes the amount of enzyme was less than in the nucleus, and it was here arranged in the form of thin granules; a small amount of RNA was also contained in the cytoplasm.

Along with the growth and maturation of the primordial follicles and their conversion, at first to young follicles and then to Graafian follicles, a lowering in the amount of RNA and alkaline phosphatase was observed in the cytoplasm of the oocyte. Infrequent clumps of RNA were localized around the nucleus of the oocytes and along the periphery of the cell, while fine granules of alkaline phosphatase were distributed throughout the cytoplasm. The nucleus of the oocyte in the young follicle contained a small amount of enzyme, distributed more frequently along the wall or to one side of the nucleolus. The nucleus of the oocyte in the Graafian follicle was free of enzyme. The nucleoli of the oocytes in the young follicles and the Graafian follicles contained much phosphatase and only a small amount of RNA.

In several of the oocytes in the Graafian follicles the nucleoli were low in alkaline phosphatase. Possibly this depended on the various stages of maturation or on some sort of functional peculiarities. In such follicles the capsules of the oocytes were rich in alkaline phosphatase, and the follicular fluid also contained a considerable amount of the enzyme but only traces of RNA.

The cytoplasm of the cells in the young yellow body contained a small amount of RNA, the clumps of which were arranged mainly around the nucleus. The nucleoli of the lutein cells contained a large amount of RNA and stained intensely with pyronine.

Alkaline phosphatase was found in the cytoplasm and nuclei of the lutein cells in the form of fine clumps. Yellow body cells were encountered with black nuclei, i.e., the activity of the enzyme here was very high; not a small number of these cells were found in which enzyme did not appear in the cytoplasm.

In the yellow body found in the stage of regression the cells became small, the amount of chromatin in the nuclei decreased, the nucleoli stained weakly with pyronine, and the RNA in the cytoplasm either completely disappeared or remained in an insignificant quantity. In both the cytoplasm and the nuclei of such a yellow body much enzyme was noted.

The alkaline phosphatase in the yellow bodies was not arranged in a single fashion; in the old yellow bodies it was in greater quantities in the nuclei, while in younger ones it was greater in the cytoplasm of the lutein cells (Fig. 1).

The administration of the cancerogenic substance over the course of 1-2 months did not cause essential changes in the function and structure of the ovaries.

After administration of orthoaminoazotoluol for $3\frac{1}{2}$ months a lowering of the body weight was observed in all the experimental animals. A reduction in the absolute and relative weight of the gonads was also noted. Thus, in the control group the weight of the two ovaries was 40.4 mg, while in the experimental group it was only

Fig. 1. Ovary of the control rat. Stained for alkaline phosphatase according to the method of Gomori. Ocular 5×, objective 4×.

Alterations in the Relationship of the Structural Components in the Ovaries of the Rat Following the Effects of Orthoaminoazotoluol Administration

| Duration of the experiment (in months | Group of animals | Admin. duration of orthoaminoazo-toluol (in months) | Weight of the body when sacrificed | Number of animals | Weight of the 2 ovaries | | Average number of structural components of the ovary oer section | | | | | Total number of structural components |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | absolute (in mg) | relative (in % | primordial follicles | mature follicles | yellow bodies | follicles containing disrupted oocytes | atretic follicles | |
| $3^1/_2$ | Control . . . . . | | 144 | 14 | 40,4 | 28,0 | 2,06 | 5,94 | 7,90 | 0,41 | 5,36 | 21,67 |
| | | | | | | | (9,7%) | (27,2%) | (36,4%) | (1,8%) | (24,9%) | (100%) |
| | Experimental . . . . . | $3^1/_2$ | 138 | 17 | 30,7 | 22,3 | 1,52 | 5,65 | 6,60 | 0,50 | 3,72 | 17,99 |
| | | | | | | | (8,4%) | (31,7%) | (36,6%) | (2,8%) | (20,5%) | (100%) |
| 7 | Control . . . . . | | 227 | 9 | 61,3 | 27,0 | 2,32 | 2,69 | 10,83 | 1,98 | 4,65 | 22,47 |
| | | | | | | | (10,1%) | (12,0%) | (48,0%) | (8,7%) | (21,2%) | (100%) |
| | Experimental . . . . . | 7 | 198 | 10 | 41,7 | 21,0 | 0,43 | 3,15 | 9,55 | 1,14 | 5,89 | 20,16 |
| | | | | | | | (1,9%) | (15,8%) | (47,4%) | (5,8%) | (29,1%) | (100%) |
| 17 | Control . . . . . | | 244 | 7 | 37,6 | 15,4 | 1,28 | 4,28 | 1,65 | 0,66 | 3,71 | 11,58 |
| | | | | | | | (11,2%) | (37,0%) | (14,7%) | (5,2%) | (31,9%) | (100%) |
| | Experimental . . . . . | 12 | 240 | 10 | 39,1 | 16,3 | 1,51 | 2,72 | 4,91 | 1,84 | 5,26 | 16,24 |
| | | | | | | | (9,3%) | (16,7%) | (30,2%) | (11,1%) | (32,7%) | (100%) |

30.7 mg. The difference is statistically valid. In this period the normal rhythm of the sexual cycle was disrupted in several of the experimental animals - the quiescent phase was prolonged. Utilizing histological investigations of these ovaries and a counting of the structural components, a decrease in the number of primordial and atretic follicles was discovered, which led to a lowering of the total number of structural components encountered per section. The processes of maturation and differentiation did not much distinguish themselves from those of the normal, which was pointed out by the count of the number of mature follicles and yellow bodies. The percent relationship of the structural components also failed to essentially change (see table).

The histochemical investigations did not demonstrate any changes in the ovaries of the experimental rats.

With the administration of the cancerogenic substance over the course of 7 months the same character of changes was preserved, but in several cases it was more clearly manifested. The body weight of the experimental animals was always lower than the body weight of the control rats. The absolute and relative weight of the ovaries in the experimental rats, despite the fact that they continued to grow, showed itself to be lower than in the control rats of that group: In the control the weight of the 2 ovaries was equal to 61.3 mg, in the experimental group 47.1 mg. The difference is statistically valid.

In these experimental intervals disturbance of ovarian function was observed in all the rats - the quiescent phase of the estral cycle was prolonged. At this time there was also encountered the most pronounced changes in the ovaries; not only was there observed a decrease in the total number of structural components seen in each section, but their percent relationship was altered as well. This appeared as a decrease in the number of primordial follicles and in an increase in the number of atretic follicles. The process of maturation and differentiation of the follicles was not disturbed, as is shown by the data on the number of mature follicles and yellow bodies (see table).

In the cells within the structural components of the ovaries of the experimental rats the character of the distribution of alkaline phosphatase and RNA was preserved, there being observed only a minimal lowering in the activity of the alkaline phosphatase and the amount of RNA, in the oocytes at various stages of their maturation, in the follicular epithelium cells, and in the yellow bodies. In isolated cases the enzyme did not appear in the follicular capsules, and only a small amount was preserved in the internal capsule of the follicle.

With prolonged administration of the orthoaminoazotoluol the ovarian capsules also lost a considerable amount of the enzyme. A reduction in the activity of alkaline phosphatase was observed in the germinative epithelium, in the nuclei of the ovarian stromal cells, and in the walls of the blood vessels (Fig. 2).

Fig. 2. Ovary of a rat after administration of orthoaminoazotoluol for 7 months. Stained for alkaline phosphatase according to the method of Gormori. Ocular 5×, objective 4×.

In the next group were included rats, which, over the course of 12 months, were fed orthoaminoazotoluol, following which the administration of the cancerogenic substance was stopped. In the ovaries of these of these animals, over the course of 5 months, the restorative processes proceeded. The absolute and relative weight of the ovaries increased. Thus, in the control group the 2 ovaries weighed 37.6 mg, while in the experimental group they weighed 39.1 mg.

The character of the ratio of structural components in the ovaries of the experimental rats in the period of restoration showed itself to be different from that in the ovaries of the control animals. These peculiarities manifested themselves by the presence of the considerable percentage of yellow bodies (in the control, 14.7%, while in the experimental group, 30.2%) and in a decrease in the percentage of maturing follicles (in the control, 37.0%, vs. only 16.7% in the experimental group). These numbers point out that in the ovaries of the control animals the alterations of aging, which usually occur at this time, were more strongly manifested, and the process of maturation and differentiation of the follicles was considerably inhibited. Atresia of the follicles occurred mainly in the intermediary stages of their development. At the same time, in the ovaries of the experimental animals during the period of restoration the process of maturation and differentiation of the follicles continued to remain intensive, and the total number of structural components in the ovaries of these rats showed itself to be greater than in the ovaries of the control animals (see table).

The increase in the number of primordial follicles in the ovaries of the rats in this group appeared as a result of their new formation. It is possible to conclude the latter on the basis of the fact that the number of primordial follicles encountered per section increased: in place of 0.43 it became 1.51. This conclusion is not altered even if one takes into consideration the fact that the weight and measurements of the ovaries of the experimental rats in this group were lower. However, the process of primordial follicle formation was not followed through by us and requires further investigation.

The activity of alkaline phosphatase and the amount of RNA in the young elements of the ovaries belonging to the experimental animals were restored to normal.

Thus, prolonged administration of orthoaminoazotoluol causes a disturbance in the function and structure of the ovaries of rats, which returns to normal after cessation of the administration of the cancerogenic substance.

## SUMMARY

An orthoaminoazotoluol solution in sunflower oil in a daily dose of 0.001 ml was given to rats with food. Following $3\frac{1}{2}$ and 7 months of the cancerogenic substance administration there was noted a decrease in the size of these organs, associated with reduced activity of alkaline phosphatase and RNA content in the cells of the ovarian structural components. The disturbances noted in the rats' ovaries disappeared after the administration of orthoaminoazotoluol was discontinued. The weight of the ovaries and the process of follicular differentiation in them normalized. Restorative processes in the ovaries were observed in 5 months.

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# THE EFFECT OF PRENATAL ASPHYXIA ON THE SIZE OF CEREBRAL NERVE CELLS

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Our previous works [1-4] showed that asphyxia, compatible with life, endured during intrauterine development, finds expression in the postnatal period in an alteration in behavior and an increase in the reactivity of the central nervous system to various irritations of the receptors and to the administration of medicinal substances in doses which do not produce particular reactions in normal animals. The alterative effect of intrauterine asphyxia is also clearly manifested by the fact that the brain of the experimental animals is always seen to be smaller in its measurements and weight than the brain of the control animals.

In the present undertaking we carried out experiments on white rats, undergoing asphyxia at different stages in their intrauterine development, studying its effect on the cerebral nerve cells.

## METHOD

In white rats pregancy lasts 21 days. Asphyxia of the pregnant animals was carried out, as in our previous experiments, by placing them in hermetically sealed chamber, the volume of which was 2 times greater than the volume of the animal. The rats were subjected to asphyxia on the 9th, 16th, and 20th day of pregnancy. The brains of the newborn rats, obtained from these animals, and also the brains from the rats surviving for 1 and 2 months after their birth, were investigated histologically.

In each age group were included 5-6 experimental animals and the same number of controls. All the animals were decapitated under the same conditions. The brains were fixed in 10% formalin, imbedded in paraffin, and stained according to the method of Nissal.

In each case, in histological sections of 8 $\mu$, the gigantic pyramid cells of the 4th field of the cerebral cortex were measured, as well as the coarse cells of the nucleus ruber and the cells of Deiter's nucleus. We also measured the dimensions of the cells of the inferior olive, which according to the hypothesis of B. N. Klosovskii [5], are inhibitory cells. For the investigation we took cells in "active condition", in which the section passed through their center, the nuclei thereby being visible and positioned in the center of the cells, and the nucleoli present.

We determined the greatest height and width of the cell body and the axes of their nuclei. We multiplied the height and width of the cell body, thus obtaining the relative area of the contoured field of the nerve cell, expressed in square microns. From the measurements of 50 cells and their nuclei we obtained an average figure for each experimental and control animal. The results were treated by the methods of variation statistics. In each age there were 5 (occasionally 6) animals; thus, from the individual values for the areas of the contoured fields of the nerve cells and their nuclei we determined the average value of the contoured field of the nerve cells and their nuclei. The results obtained were again treated by the methods of variation statistics. The same calculations were carried out for the control animals as well.

The results obtained are presented in Tables 1, 2, and 3.

From the data in Table 1, 2, and 3 it is apparent that asphyxia endured in the embryonal period leads to a considerable decrease in the measurements of the nerve cells and their nuclei (contoured field). This decrease is manifestly clearer in those cases where the fetuses underwent asphyxia in earlier stages of their intrauterine development.

## SUMMARY

The author studied the effect of prenatal asphyxia on the size of cerebral cells. Experiments were conducted on white rats which sustained asphyxia on the 9th, 16th, and 20th days of pregnancy. The brain of the newborn ratlings obtained from these animals, as well as the brain of the 1 and 2 months-old rats was subjected to histological examination. Nissl's method was used for the treatment of the preparations. The author measured the greatest height and the width of the cellular body and of the axis of cellular nuclei, the giant pyramids of the 4th field of cerebral cortex, the large cells of the cellular body and of the axis of their nuclei, the giant pyramids of the 4th field of cerebral cortex, the large cells of the nucleus ruber and of the Deiters' nucleus, as well as the cells of the inferior olive. For each experimental and control animal an average figure for 50 measurement of the cells and their nuclei was estimated. The results were processed by the method of variation statistics. There were 5, at

TABLE 1. Decrease in the Measurements of the Nerve Cells and Their Nuclei in Newborn Rats Which Endured Prenatal Asphyxia at Various Intervals During the Pregnancy

| No. | Nucleus | Experimental rats. Born 5 days after asphyxia | Standard error | Experimental rats. Born 12 days after asphyxia | Standard error | Control | Standard error |
|---|---|---|---|---|---|---|---|
| | Product of the height times the width of the nerve cells (contoured field) in $\mu^2$ | | | | | | |
| 1 | 4th field | 146,80 | ±0,21 | 146,21 | ±0,20 | 221,65 | ±0,21 |
| 2 | Nucleus ruber | 277,97 | ±0,17 | 272,48 | ±0,21 | 358,30 | ±0,27 |
| 3 | Deiter's nucleus | 474,85 | ±0,31 | 423,46 | ±0,28 | 849,41 | ±0,44 |
| 4 | Inferior olives | 186,69 | ±0,21 | 176,86 | ±0,21 | 239,24 | ±0,22 |
| | Product of the axes of the nuclei (contoured field) in $\mu^2$ | | | | | | |
| 1 | 4th field | 95,09 | ±0,18 | 85,75 | ±0,16 | 126,22 | ±0,14 |
| 2 | Nucleus ruber | 162,22 | ±0,17 | 142,94 | ±0,18 | 215,05 | ±0,17 |
| 3 | Deiter's nucleus | 208,63 | ±0,17 | 170,09 | ±0,25 | 319,54 | ±0,21 |
| 4 | Inferior olives | 114,48 | ±0,20 | 109,60 | ±0,17 | 150,63 | ±0,19 |

TABLE 2. Decrease in the Measurements of the Nerve Cells and Their Nuclei in 1 Month Old Rats Which Endured Prenatal Asphyxia at Various Intervals During the Pregnancy

| No. | Nucleus | Experimental rats. Born on the following day after asphyxia | Standard error | Experimental rats. Born 12 days after asphyxia | Standard error | Experimental rats. Born 12 days after asphyxia | Standard error | Control | Standard error |
|---|---|---|---|---|---|---|---|---|---|
| | Product of the height times the width of the nerve cell (contoured field) in $\mu^2$ | | | | | | | | |
| 1 | 4th field | 541,22 | ±0,28 | 395,38 | ±0,38 | 296,48 | ±0,15 | 622,35 | ±0,26 |
| 2 | Nucleus ruber | 715,66 | ±0,33 | 594,04 | ±0,29 | 485,71 | ±0,34 | 1083,78 | ±0,52 |
| 3 | Deiter's nucleus | 1073,74 | ±0,68 | 920,69 | ±0,56 | 780,78 | ±0,38 | 1234,30 | ±0,65 |
| 4 | Inferior olives | 208,88 | ±0,25 | 255,52 | ±0,14 | 251,16 | ±0,14 | 327,26 | ±0,38 |
| | Product of the axes of the nuclei contoured field) in $\mu^2$ | | | | | | | | |
| 1 | 4th field | 180,94 | ±0,15 | 165,86 | ±0,15 | 105,12 | ±0,18 | 254,02 | ±0,14 |
| 2 | Nucleus ruber | 168,98 | ±0,20 | 215,02 | ±0,13 | 160,50 | ±0,15 | 278,66 | ±0,15 |
| 3 | Deiter's nucleus | 303,06 | ±0,23 | 221,49 | ±0,21 | 188,88 | ±0,16 | 315,10 | ±0,19 |
| 4 | Inferior olives | 123,36 | ±0,17 | 120,14 | ±0,14 | 102,54 | ±0,14 | 165,81 | ±0,16 |

TABLE 3. Decrease in the Measurements of the Nerve Cells and Their Nuclei in 2 Months Old Rats Which Endured Prenatal Asphyxia at Various Intervals During the Pregnancy

| No. | Nucleus | Experimental rats. Born on the following day after asphyxia | Standard error | Experimental rats. Born 12 days after asphyxia | Standard error | Experimental rats. Born 19 days after asphyxia | Standard error | Control | Standard error |
|---|---|---|---|---|---|---|---|---|---|
| | Product of the height times the width of the nerve cell (contoured field) in $\mu^2$ | | | | | | | | |
| 1 | 4th field | 658,69 | ±0,27 | 524,26 | +0,29 | 468,62 | ±0,26 | 712,30 | ±0,17 |
| 2 | Nucleus ruber | 870,34 | ±0,43 | 866,98 | ±0,20 | 760,58 | ±0,61 | 1095,48 | ±0,37 |
| 3 | Deiter's nucleus | 1088,32 | ±0,33 | 966,15 | ±0,27 | 812,96 | ±0,48 | 1257,22 | ±0,59 |
| 4 | Inferior olives | 298,12 | ±0,15 | 289,36 | ±0,23 | 263,63 | ±0,18 | 340,02 | ±0,24 |
| | Product of the axes of the nuclei contoured field) in $\mu^2$ | | | | | | | | |
| 1 | 4th field | 281,60 | +0,17 | 224,56 | ±0,56 | 203,48 | ±0,16 | 289,39 | ±0,15 |
| 2 | Nucleus ruber | 265,30 | ±0,22 | 232,92 | ±0,17 | 226,21 | ±0,20 | 291,86 | ±0,22 |
| 3 | Deiter's nucleus | 306,32 | ±0,22 | 248,22 | ±0,28 | 233,56 | ±0,27 | 321,27 | ±0,25 |
| 4 | Inferior olives | 145,54 | ±0,12 | 120,86 | ±0,16 | 108,03 | ±0,16 | 183,56 | ±0,15 |

times 6 animals of each age and therefore the averages for the cross section area of the nerve cells and their nuclei were estimated from values of individual areas. The results obtained were again processed by the variation statistics method. The same estimations were made in the instance of control animals. The above demonstrated that prenatal asphyxia leads to a considerable decrease in the size of the nerve cells and their nuclei (cross section). This reduction was more clear-cut when the embryo was subjected to asphyxia at the earlier stages of the intrauterine development.

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# MORPHOLOGICAL EVIDENCE FOR DOUBLE INNERVATION OF NEURONS OF PERIPHERAL GANGLIA

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In earlier publications [2, 3, 4], reporting results of our investigations and evidence from the literature, it was indicated that neurons of peripheral ganglia of the vegetative nervous system make synaptic connection not only with centrally originating preganglionic nerve cells, but also with axons of peripheral sensory neurons. In other words, peripheral ganglia are not only cross-over points for impulses, following the activity of the central nervous system, but also peripheral reflex centers.

It is important to examine the topographical relations of synaptic formations of preganglionic cells and axons of peripheral sensory neurons.

In one of the cited works [2] it was shown that section of N. intestinalis results in degeneration of ascending axons of Dogiel type II cells in the central regions of these nerves, and degeneration of the pericellular networks formed by them in the solar plexus. In addition it was shown that on individual neurons it is possible to detect degenerating pericellular networks and intact synaptic boutons. A similar observation is also reported by I. A. Chervova [5], indicating that after bilateral vagotomy, neurons of the intramural ganglia of the right auricle exhibit degenerating and intact pericellular networks.

The purpose of the present investigation was to study this problem in experiments permitting analysis of the morphological substrate of subordination which relates the central nervous system and peripheral reflex mechanisms.

## METHOD

We conducted three series of experiments on cats (male and female): 1) Unilateral and bilateral section of pelvic nerves with subsequent examination of ganglia of pelvic organs – uterus (cervical ganglion prostate gland, urethra, urinary bladder, rectum; 2) Total removal of the spinal cord according to our own method [4] and examination of ganglia of the pelvis and peritoneal cavity; 3) Bilateral vagotomy and examination of the intramural ganglia of the heart. Animals were sacrificed 2,3, 4, and 5 days after operation. Material was prepared by silver methods: Campos, Bielschowsky – Gros (Lavrient'ev modification), and Cajal.

## RESULTS

Massive degeneration of preganglionic nerve fibers and of the pericellular networks formed by them was observed 3-4 days following nerve section or removal of the spinal cord. As a result of degeneration of synaptic ringlets, boutons become homogenous forms and decompose into intensively impregnating globules. The pericellular terminal filaments which are adjacent to the cell body, or dendrites, and which form the intercellular plexus but not special terminal structures, at first acquire an intensive, black, varicose character, and degenerate into separate fragments and globules.

Intact synapses occur in ganglia along with degenerating pericellular structures. As was shown in chronic experiments involving removal of the spinal cord, synapses remain preserved indefinitely.

Thus, these experiments have confirmed earlier observations concerning the presence of synaptic connections between peripheral sensory cells (Dogiel type II) and effector neurons. These synaptic connections are accomplished by delicate axon terminal filaments entwining the soma of the nerve cell.

The terminal filaments which form the pericellular network may also cross over to other neurons or terminate in the intercellular plexus of the ganglia, where they make intimate contact with ramifications of dendrites of effector neurons and sometimes exhibit localized coils or varicose swelling [3, 4].

The correlation between degenerating and intact pericellular networks, examination of which was initiated in the present investigation, deserves separate consideration.

We were not able to show major structural differences between synaptic connections of neurons of different ganglia; however, in all examined ganglia the most typical picture is the presence on one and the same nerve cell of intact and degenerating pericellular networks from specific nerve fibers. Degenerating terminal structures also occur in the intercellular ganglionic plexus, i.e., in the terminology of certain authors, it is possible to speak of degeneration of axosomatic and axodendritic synapses of ganglia, if these terms really reflect both morphology and function.

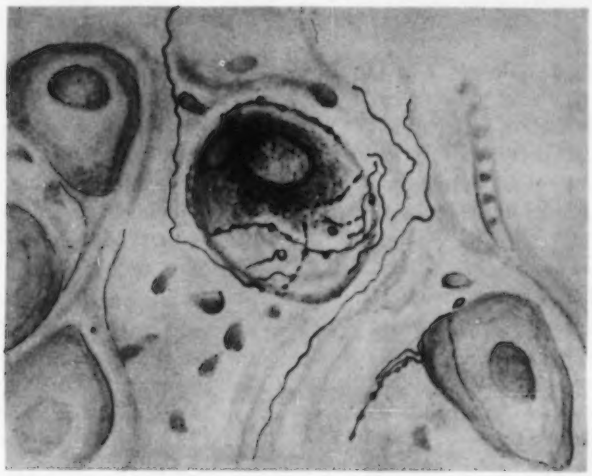

Fig. 1. Degenerating pericellular network and intact synapses in solar plexus after section of N. intestinalis.

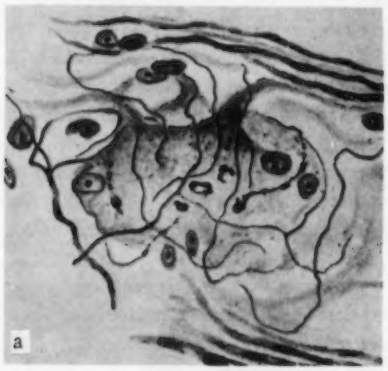

Fig. 2. "Double" synapses in intramural ganglia. a) Degenerating and intact pericellular networks and synapses of neurons of the intramural ganglia of the urinary bladder, after section of the left pelvic nerve; b) nerve cells of the intramural ganglia of the urinary bladder with degenerating and intact synapses after section of the 1st pelvic nerve. (Small arrows mark contact of some endings with glial cells.)

Changes following section of N. intestinalis in the solar plexes (Fig. 1) were similar to those observed in the small urinary bladder ganglion – simultaneous representation on one and the same nerve cell of degenerating synapses and intact pericellular filaments (Fig. 2a).

Analysis of the major number of preparations revealed another, independently important fact: Neurons of peripheral ganglia do not receive equivalent innervation (at least according to the morphological picture from centrally originating preganglionic fibers and from axons of Dogiel type II cells.

In addition to individual neurons exhibiting the changes described above, there occur neurons which show only degenerating synapses, i.e., ending of centrally originating preganglionic fibers, and others with only intact pericellular networks without degenerating synapses. However, the majority of neurons in ganglia examined by us exhibit "double" synaptic connections. Along with this it was found that on individual neurons pericellular networks of various origins are not represented in the same degree. In one case there is greater development of synaptic structures of centrally originating preganglionic fibers; in the other – only one or two terminal boutons may occur, along with an intact pericellular network of terminal axon filaments which entwine the soma of the neuron and attain considerable development. The different forms of "double" synaptic connection of peripheral neurous are schematized in Fig. 3.

It is important to point out that following unilateral section of pelvic nerves, individual cells with degenerating and preserved preganglionic synaptic terminations occur in one and the same ganglion (Fig. 2b); in this same figure one can see degenerating and intact synaptic boutons on one nerve cell.

These observations suggest the possibility of overlapping synaptic endings of preganglionic fibers of right and left pelvic nerves in one and the same ganglion and on one and the same neuron. Figure 2b is a good illustration of the intimate contact of some synaptic terminations with glial cells.

All interpretations indicate the following: Besides the universally known fact that the multiplicity of synaptic endings [1] results in overlapping terminations of different preganglionic nerve fibers with individual peripheral neurons, the latter are also connected with axons of peripheral sensory neurons. Consequently, interneuron endings in peripheral ganglia of the vegetative nervous system are considerably more complex than the descriptions resulting from the work of Langley.

In discussing the results of our investigations, one should compare the evidence from the literature, according to which neurons of the central nervous system exhibit endings from afferent nerve fibers of various origins; in particular, such endings occur in motoneurons of the spinal cord [10, etc.].

Some of the available material from physiological investigations can be used for a physiological interpretation of our observations. Such an interpretation involves the concept of convergence of nerve impulses, which plays an important role in analyses of the activity of neural centers and also of peripheral ganglia. Thus, Amassian and De Vito [6], Hernandez-Peon and Hagbarth [9], in electrophysiological studies, showed that an individual cell of the reticular formation of the brainstem can simultaneously receive impulses from different afferent systems or from cortical and cerebellar regions [11]. Convergence of impulses, according to a series of authors, plays an important role in the corrdination of activity and the spinal cord [12].

Directly related to the discussion are the investigations of Eccles [8], Bronk [7], etc., according to which convergence of impulses is an essential mechanism in the activity of vegetative ganglia.

Investigators believe that summation is possible during collision of impulses on individual neurons, and that this results in excitation and facilitation of conduction; if these same impulses are blocked, inhibition of the neuron occurs.

It becomes obvious that analysis of the central influence on organs must account for interaction of impulses entering ganglia through preganglionic fibers on the one hand, and impulses entering through axons of peripheral sensory neurons on the other hand. Peripheral sensory neurons are stimulated by excitation of receptor endings of their processes; in each case the intensity of such stimulation signals the functional state of the organ (e.g., degree of overfilling of the organ area, etc.).

Concerning the reticular formation, the work cited also indicates that overlapping of different afferent systems on individual neurons is not absolute. Neurons are

Fig. 3. Schematic illustration of types of "double" synapatic endings on neurons of peripheral ganglia.

found which are primarily dependent on one or a group of afferent systems (e.g., in one case, proprioceptive and cutaneous; in another, cutaneous and auditory; in a third, exclusively auditory; etc.).

Thus, there is a basis for our descriptions of structural relations (involving innervation of peripheral neurons by centrally originating preganglionic fibers and axons of peripheral sensory neurons) to be considered as particular cases in the structural and functional organization of the nervous system.

## SUMMARY

In experiments on cats synaptic connections of the effector neurons in the peripheral ganglia of the abdominal and pelvic organs and heart were studied by the methods of experimental neuromorphology. Experimental evidence obtained confirm the data presented in the previously published works on the synaptic connections of the effector neurons with the axons of the peripheral sensory neurons. The preganglionic fibers terminate with the typical end-loops of free terminal fibers. The synaptic connection between the peripheral sensory neurons is effected at the expense of the terminal fibers distributed in the intercellular network, and entering into an intimate association with the dendrites and the cellular bodies. Double synaptic connection is found in the majority of the neurons. The morphological pictures point to an uneven innervation of the individual neurons by the preganglionic fibers and axons of the peripheral sensory neurons. Physiological investigations respective convergence of nerve impulses on individual neurons give an approach to the understanding of the mechanism governing the CNS subordinating influences on the peripheral reflexes mechanisms.

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| Abbreviation* | Journal* | Translation |
|---|---|---|
| Agrobiol. | Agrobiologiya | Agrobiology |
| Akusherstvo i Ginekol. | Akusherstvo i Ginekologiya | Obstetrics and Gynecology |
| Antibiotiki | Antibiotiki | Antibiotics |
| Aptechnoe Delo | Aptechnoe Delo | Pharmaceutical Transactions |
| Arkh. Anat. Gistol. i Émbriol. | Arkhiv Anatomii Gistologii i Émbriologii | Archives of Antomy, Histology, and Embryology |
| Arkh. Biol. Nauk SSSR | Arkhiv Biologicheskikh Nauk SSSR | Archives of Biological Science USSR |
| Arkh. Patol. | Arkhiv Patologii | Archives of Pathology |
| Biofizika | Biofizika | Biophysics |
| Biokhimiya | Biokhimiya | Biochemistry |
| Biokhim. Plodov i Ovoshchei | Biokhimiya Plodov i Ovoshchei | Biochemistry of Fruits and Vegetables |
| Bot. Zhur. | Botanicheskii Zhurnal | Journal of Botany |
| Byull. Éksptl. Biol. i Med. | Byulleten Éksperimentalnoi Biologii i Meditsiny | Bulletin of Experimental Biology and Medicine |
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| Doklady Akad. Nauk SSSR | Doklady Akademii Nauk SSSR | Proceedings of the Academy of Sciences USSR |
| Éksptl. Khirurg. | Éksperimentalnaya Khirurgiya | Experimental Surgery |
| Farmakol. i Toksikol. | Farmakologiya i Toksikologiya | Pharmacology and Toxicology |
| Farmatsiya | Farmatsiya | Pharmacy |
| Fiziol. Rastenii | Fiziologiya Rastenii | Plant Physiology |
| Fiziol. Zhur. SSSR | Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova | I. M. Sechenov Physiology Journal USSR |
| Gigiena i Sanit. | Gigiena i Sanitariya | Hygiene and Sanitation |
| Izvest. Akad. Nauk SSSR, Ser. Biol. | Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya | Bulletin of the Academy of Sciences USSR, Biology Series |
| Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog. | Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii | Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography |
| Khirurgiya | Khirurgiya | Surgery |
| Klin. Med. | Klinicheskaya Meditsina | Clinical Medicine |
| Lab. Delo | Laboratornoe Delo (po Voprosam Meditsiny) | Laboratory Work (on Medical Problems) |
| Med. Parazitol. | Meditsinskaya Parazitologiya i Parazitarnye Bolezni | Medical Parasitology and Parasitic Diseases |
| Med. Radiol. | Meditsinskaya Radiologiya | Medical Radiology |
| Med. Zhur. Ukrain. | Medichnii Zhurnal Ukrainskii | Ukrainian Medical Journal |
| Mikrobiologiya | Mikrobiologiya | Microbiology |
| Mikrobiol. Zhur. | Mikrobiologichnii Zhurnal | Microbiology Journal |
| Nevropatol., Psikhyat. i Psikhogig. | Nevropatologiya, Psikhyatriya i Psikhogigiena | Neuropathology, Psychiatry and Psychohygiene |
| Ortoped., Travmatol. i Protez. | Ortopediya, Travmatologiya i Protezirovanie | Orthopedics, Traumatology and Prosthetics |
| Parazitol. Sbornik | Parazitologicheskii Sbornik | Parasitology Collection |
| Pediatriya | Pediatriya | Pediatrics |
| Pochvovedenie | Pochvovedenie | Soil Science |
| Priroda | Priroda | Nature |
| Problemy Éndokrinol. i Gormonoterap. | Problemy Endokrinologii i Gormonoterapii | Problems of Endocrinology and Hormone Therapy |
| Problemy Gematol. | Problemy Gematologii i Perelivaniya Krovi | Problems of Hematology and Blood Transfusion |
| Problemy Tuberk. | Problemy Tuberkuleza | Problems of Tuberculosis |
| Sovet. Med. | Sovetskaya Meditsina | Soviet Medicine |
| Sovet. Vrachebny Zhur. | Sovetskii Vrachebnyi Zhurnal | Soviet Physicians Journal |
| Stomatologiya | Stomatologiya | Stomatology |

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

(continued)

| Abbreviation | Journal | Translation |
|---|---|---|
| Terap. Arkh. | Terapevticheskii Arkhiv | Therapeutic Archives |
| Trudy Gelmint. Lab. | Trudy Gelmintologicheskoi Laboratoriya | Transactions of the Helminthology Laboratory |
| Trudy Inst. Genet. | Trudy Instituta Genetiki | Transactions of the Institute of Genetics |
| Trudy Inst. Gidrobiol. | Trudy Instituta Gidrobiologiya | Transactions of the Institute of Hydrobiology |
| Trudy Inst. Mikrobiol. | Trudy Instituta Mikrobiologiya | Transactions of the Institute of Microbiology |
| Trudy Inst. Okean. | Trudy Instituta Okeanologiya, Akademii Nauk SSSR | Transactions of the Institute of Oceanology, Academy of Sciences, USSR |
| Trudy Leningrad Obshchestva Estestvoisp. | Trudy Leningrad Obshchestva Estestvoispytatelei | Transactions of the Leningrad Society of Naturalists |
| Trudy Vsesoyuz. Gidrobiol. Obshchestva | Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva | Transactions of the All-Union Hydrobiological Society |
| Trudy Vsesoyuz. Inst. Ekspti. Med. | Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny | Transactions of the All-Union Institute of Experimental Medicine |
| Ukrain. Biokhim. Zhur. | Ukrainskii Biokhimichnii Zhurnal | Ukrainian Biochemical Journal |
| Urologiya | Urologiya | Urology |
| Uspekhi Biokhimiya | Uspekhi Biokhimiya | Progress in Biochemistry |
| Uspekhi Sovremennoi Biol. | Uspekhi Sovremennoi Biologiya | Progress in Contemporary Biology |
| Vestnik Akad. Med. Nauk SSSR | Vestnik Akademii Meditsinskikh Nauk SSSR | Bulletin of the Academy of Medical Science USSR |
| Vestnik Khirurg. im. Grekova | Vestnik Khirurgii imeni Grekova | Grekov Bulletin of Surgery |
| Vestnik Leningrad. Univ. Ser. Biol. | Vestnik Leningradskogo Universiteta, Seriya Biologii | Journal of the Leningrad Univ., Biology Series |
| Vestnik Moskov. Univ., Ser. Biol. i Pochvov. | Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya | Bulletin of the Moscow University, Biology and Soil Science Series |
| Vestnik Oftalmol. | Vestnik Oftalmologii | Bulletin of Ophthalmology |
| Vestnik Oto-rino-laringol. | Vestnik Oto-rino-laringologiya | Bulletin of Otorhinolaryngology |
| Vestnik Rentgenol. i Radiol. | Vestnik Rentgenologii i Radiologii | Bulletin of Roentgenology and Radiology |
| Vestnik Venerol. i Dermatol. | Vestnik Venerologii i Dermatologii | Bulletin of Venereology and Dermatology |
| Veterinariya | Veterinariya | Veterinary Science |
| Vinodelie i Vinogradarstvo | Vinodelie i Vinogradarstvo SSSR | Wine-Making and Viticulture |
| Voprosy Klin. | Voprosy Klinicheskie | Clinical Problems |
| Voprosy Med. Khim. | Voprosy Meditsinskoi Khimii | Problems of Medical Chemistry |
| Voprosy Med. Virusol. | Voprosy Meditsinskoi Virusologii | Problems of Medical Virology |
| Voprosy Neirokhirurg. | Voprosy Neirokhirugii | Problems of Neurosurgery |
| Voprosy Onkol. | Voprosy Onkologii | Problems of Oncology |
| Voprosy Pitaniya | Voprosy Pitaniya | Problems of Nutrition |
| Voprosy Psikhologii | Voprosy Psikhologii | Problems of Psychology |
| Voprosy Virusologii | Voprosy Virusologii | Problems of Virology |
| Vrachebnoe Delo | Vrachebnoe Delo | Medical Profession |
| Zav. Lab. | Zavodskaya Laboratoriya | Factory Laboratory |
| Zhur. Mikrobiol., Epidemiol. i Immunobiol. | Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii | Journal of Microbiology, Epidemiology, and Immunobiology |
| Zhur. Nevropatol. i Psikhiat. | Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov | S. S. Korsakov Journal of Neuropathology and Psychiatry |
| Zhur. Obshchei Biol. | Zhurnal Obshchei Biologiya | Journal of General Biology |
| Zhur. Vysshei Nerv. Deyatel. | Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova | I. P. Pavlov Journal of Higher Nervous Activity |
| Zool. Zhur. | Zoologicheskii Zhurnal | Journal of Zoology |

## SIGNIFICANCE OF ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN SOVIET PERIODICALS

| | |
|---|---|
| FIAN | Phys. Inst. Acad. Sci. USSR. |
| GDI | Water Power Inst. |
| GITI | State Sci.-Tech. Press |
| GITTL | State Tech. and Theor. Lit. Press |
| GONTI | State United Sci.-Tech. Press |
| Gosenergoizdat | State Power Press |
| Goskhimizdat | State Chem. Press |
| GOST | All-Union State Standard |
| GTTI | State Tech. and Theor. Lit. Press |
| IL | Foreign Lit. Press |
| ISN (Izd. Sov. Nauk) | Soviet Science Press |
| Izd. AN SSSR | Acad. Sci. USSR Press |
| Izd. MGU | Moscow State Univ. Press |
| LEIIZhT | Leningrad Power Inst. of Railroad Engineering |
| LET | Leningrad Elec. Engr. School |
| LETI | Leningrad Electrotechnical Inst. |
| LETIIZhT | Leningrad Electrical Engineering Research Inst. of Railroad Engr. |
| Mashgiz | State Sci.-Tech. Press for Machine Construction Lit. |
| MEP | Ministry of Electrical Industry |
| MES | Ministry of Electrical Power Plants |
| MESEP | Ministry of Electrical Power Plants and the Electrical Industry |
| MGU | Moscow State Univ. |
| MKhTI | Moscow Inst. Chem. Tech. |
| MOPI | Moscow Regional Pedagogical Inst. |
| MSP | Ministry of Industrial Construction |
| NII ZVUKSZAPIOI | Scientific Research Inst. of Sound Recording |
| NIKFI | Sci. Inst. of Modern Motion Picture Photography |
| ONTI | United Sci.-Tech. Press |
| OTI | Division of Technical Information |
| OTN | Div. Tech. Sci. |
| Stroiizdat | Construction Press |
| TOE | Association of Power Engineers |
| TsKTI | Central Research Inst. for Boilers and Turbines |
| TsNIEL | Central Scientific Research Elec. Engr. Lab. |
| TsNIEL-MES | Central Scientific Research Elec. Engr. Lab.– Ministry of Electric Power Plants |
| TsVTI | Central Office of Economic Information |
| UF | Ural Branch |
| VIESKh | All-Union Inst. of Rural Elec. Power Stations |
| VNIIM | All-Union Scientific Research Inst. of Meteorology |
| VNIIZhDT | All-Union Scientific Research Inst. of Railroad Engineering |
| VTI | All-Union Thermotech. Inst. |
| VZEI | All-Union Power Correspondence Inst. |

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. – Publisher.

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